LINKING A TOXIC GLUCOSE METABOLITE TO GLYCEMIC AND CARDIOVASCULAR RESPONSES IN AN OMNIVORE COMPARED TO A CARNIVORE

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By

JENNIFER BRIENS

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ABSTRACT

Pet nutrition is becoming increasingly important to pet owners with more owners claiming that they are conscious of the ingredients in their pet foods. Healthy whole nutritious pet foods are important to pet owners although some of the health claims made by pet food companies do not have a scientific basis. High protein costs are a common driver for the pet food industry to formulate their foods with increased levels of carbohydrates. Cats have different nutritional requirements, and being a carnivorous species, are thought to handle high levels of ingested carbohydrates poorly. The purpose of this thesis was to determine how glycemic index values differed for various starches and diets in a carnivorous species, cats, and an omnivorous species, dogs. With this novel information, low glycemic index diets were formulated using either modified cornstarch or pulse starches (pea, lentil or faba bean) as carbohydrate sources. I aimed to provide long-term health benefits to dogs and cats through feeding low glycemic index diets that would control postprandial glycemic and insulinemic responses, plus reduce postprandial levels of the toxic glucose metabolite, methylglyoxal, and promote cardiovascular health.

Two studies were designed to determine the research objectives:

- Determine species metabolic differences (dog versus cat) to both low and high glycemic index carbohydrate sources, and link these to postprandial cardiovascular and methylglyoxal results; and
- Compare long-term health benefits of diets formulated with pulse starch to a modified cornstarch diet in dogs versus cats, including digestibility, glycemic, insulinemic, and cardiovascular responses, and postprandial methylglyoxal levels.

For the first study the glycemic index was determined for nine different starch sources and four whole formulated diets in laboratory beagles and domestic, mixed breed cats. For this first study, I related the postprandial glycemic and insulinemic responses to methylglyoxal and cardiovascular responses after single feedings in animals not previously acclimated to the test diets. The results from this study showed that pulse starches produce lower GI values in both dogs and cats when compared to common pet food ingredients such as unmodified corn, rice flour and white wheat flour, and other starch sources such as tapioca, modified cornstarch, and potato. However, in dogs only, the potato starch produced the lowest GI value. When modified cornstarch versus pulse starches (pea, lentil or faba bean) were used at a 30% inclusion rate in whole diets, glycemic responses increased in both species, likely due to formulation and processing effects. Contradictory to what was initially thought, cats can handle higher amounts of carbohydrates in their diets which is shown by the low glycemic responses and high digestibility of the pure starches and whole diets. Postprandial increases in plasma glucose were linked with increased levels of methylglyoxal in dogs. In contrast, in cats, there was no association between glycemic index and postprandial methylglyoxal changes. Interestingly methylglyoxal decreases from pre-feeding to 60 minutes postprandial were observed for most of the starches and whole diets. Contradictory to the acute study, the long-term study results showed that this link between postprandial glycemic response and methylglyoxal is not supported in dogs. The long-term study results in the cats agree with the acute study in that there was no link between postprandial methylglyoxal levels and glycemic index. The results from this study support the use of low glycemic pulse carbohydrate sources in pet food, which can now be marketed as low glycemic index food using scientific support. I also showed that cats are able to

control postprandial blood glucose levels following consumption of multiple starch sources, contradictory to what has been previously reported in the literature.

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figures Page

LIST OF ABBREVIATIONS

AGEs – Advanced glycation end products

AUC – Area under the curve

CBC – Complete blood count

DE – Digestible energy

DM – Dry matter

ELISA – Enzyme linked immunosorbent assay

GI – Glycemic Index

IVGGT – Intravenous glucose tolerance test

LSD – Least significant difference

M-mode – Motion mode

ME – Metabolizable energy

MG – Methylglyoxal

NT – Nitrotyrosine

OGTT – Oral glucose tolerance test

ROS – Reactive oxygen species

TTAD – Total tract apparent digestibility

1. RATIONALE, OBJECTIVES AND HYPOTHESES

1.1. Rationale

The pet food industry is constantly changing food formulations to meet a balance between what animals nutritionally need and what consumers want. A nutritious, whole formulated diet is necessary for a healthy life and longevity of our pets. Cats, being carnivores, have different nutritional requirements than dogs, which are omnivorous like humans. It is a common misconception that cats can be fed similar to a small dog, but they require greater amounts of specific amino acids and vitamins such as taurine, arginine, methionine, vitamin A, and thiamine in their diets than in canine diets. Along with having specific amino acid requirements, cats are hypothesized to be less able to utilize moderate to high levels of dietary carbohydrates (Zoran. 2002; Farrow et al. 2013), which can result in postprandial increases in glucose and associated toxic metabolites. However, much of this assumption may be unproven and based on the high protein diet of feral or wild cats (Plantinga et al. 2011). Dietary changes with domestication to higher carbohydrate diets are thought to contribute to increases in obesity, insulin resistance and diabetes in both dogs and cats (Rand et al. 2004). Carbohydrates are the main nutrient responsible for altering postprandial glucose and insulin responses in dogs, but this may not be true for cats (Verbrugghe et al. 2010). Based on human nutrition, pulses such as peas, lentils and faba beans are considered low glycemic index (GI) foods compared to grains such as corn and rice. Consuming a diet with high GI foods can lead to hyperglycemia which is known to negatively affect endothelial function (Loader et al. 2015). Hyperglycemia has also been shown to be linked to the production of a toxic reactive glucose metabolite, methylglyoxal (MG), which has been shown to increase oxidative stress (Desai et al. 2010) and inflammation (Dhar et al.

2008) and can lead to endothelial dysfunction (Dhar et al. 2010) which can negatively impact cardiovascular health. Therefore, the overall goal of this thesis was to determine if pulses are a healthier alternative carbohydrate source in pet food than cereals, in terms of both short and long-term health in both dogs and cats.

1.2. Research Design

For this thesis, I determined the digestibility of the starch sources in diets to evaluate if lower digestibility results in lower postprandial glucose responses which is reported to be true in humans. Next, I evaluated postprandial metabolic and cardiovascular responses to a fairly comprehensive selection of potential carbohydrate sources in pet foods, then linked these to postprandial changes in methylglyoxal. Macronutrient content effects on metabolic parameters is a controversial topic, especially in cats. For both dogs and cats some studies report that high carbohydrate levels induce high postprandial glucose or insulin responses (Nguyen et al. 1998; Elliott et al. 2012; Farrow et al. 2013) whereas other studies showed either fat or protein as being responsible for inducing high postprandial glucose or insulin responses (Thiess et al. 2004; Backus et al. 2007; Verbrugghe et al. 2010). Not only does the macronutrient content affect metabolic responses, but the type of carbohydrate used in the diet can play a role as well (Carciofi et al. 2008; de Oliveira et al. 2008). Very few studies, to our knowledge, have evaluated postprandial responses to feedings of only the carbohydrate source alone and to using pulses as the carbohydrate source in food. No studies have evaluated species comparisons (dog vs cat) to pulse-based diets for either acute or chronic feedings. I determined both acute and chronic health effects of pulse-based diets in dogs and cats. The first study performed was the acute single feedings of nine pure starch sources and four whole formulated diets in beagles (n=8; obtained from Marshall Farms, NY) or domestic, mixed breed cats (n=8-9; obtained from

Kansas State University, Kansas City, MO) not previously acclimated to the test diets. I examined the responses of these nine starch sources (modified cornstarch, unmodified cornstarch, pea, faba bean, lentil, rice flour, tapioca, white wheat flour and potato) to expand on previous work done in our lab (Adolphe et al. 2012) and determine area under the curve (AUC) values for glycemic and insulinemic responses and glycemic index values for more starch sources in both dogs and cats. Area under the curve uses the trapezoid rule to calculate the glycemic response area of the curve for calculation of glycemic index (Wolever et al. 1991). Corn and rice were chosen because they are typical carbohydrates that are found in pet food and in humans are considered high glycemic index starches. I examined three pulse crops, pea, lentil and faba bean because in humans these are low glycemic index carbohydrate options (Foster-Powell et al. 1995) and in dogs, peas have previously been reported to have a low glycemic index (Adolphe et al. 2012 & 2015). Tapioca starch, white wheat flour and potato starch were added to the study as other commonly used pet food ingredients to expand on the current knowledge of glycemic index values in dogs and cats. Pulses, in particular peas, are becoming a more common ingredient as an alternative option to corn and rice in dog food (Pet Food Industry. 2017), but scientific knowledge of the benefits of pulses in cat food are lacking. Saskatchewan is one of the top producers of pulse grains in the world so expanding on another market for pulses can also have economic benefits (Saskatchewan Pulse Growers. 2017). I also looked at the glycemic index values of whole formulated diets containing modified cornstarch, pea, lentil or faba bean as the starch source. Modified cornstarches are chemically or enzymatically altered and produced to enhance certain physiochemical properties of the starch, such as viscosity, gelling, or even the digestibility (Chung et al. 2008). I assessed both

unmodified and modified cornstarch for the acute feedings of the starches, but for the diets I only looked at modified cornstarch.

The second, long-term study was done to look at chronic health effects of feeding the four formulated diets (30% inclusion of pea, lentil or faba bean starch versus modified cornstarch) This study is relevant to real world situations since pets normally consume the same diet for a good portion of their lives. The animals were fed one of the four diets for six weeks in a crossover study design, with two weeks washout on a normal commercial diet between each test period. During the six weeks, weight and food intake were monitored on a weekly basis. The animals were tested at the end of each feeding period for glycemic and insulinemic responses to a glucose control or a meal of the diet being tested. I also examined postprandial cardiovascular responses, changes in MG blood levels and a plasma indicator of oxidative stress (nitrotyrosine).

1.3. Objectives

The overall objective of this project was to expand on the existing literature for glycemic index values of various carbohydrate sources for both dogs and cats and also determine if this methodology is suitable for companion animals. I also determined potential health benefits of pulse starches for both acute and chronic consumption. This was done by evaluating postprandial changes in plasma glucose, insulin, methylglyoxal (MG), nitrotyrosine (NT) and cardiovascular responses in an omnivorous species compared to carnivorous species to carbohydrates.

Study 1: Acute Postprandial Responses to Starches and Whole Diets in Dogs and Cats

The objective of the first study was to compare glycemic, insulinemic and cardiovascular responses to single feedings of nine starch sources and four whole formulated diets in dogs and cats not previously acclimated to the test diets, then link these glycemic responses to

postprandial changes in levels of MG. From these I determined glycemic index values for the carbohydrate components of pet foods for dogs and cats.

Study 2: Long-term Health Benefits of Pulse Verses Corn-Based Formulated Diets

The objective of the second study was to determine the digestibility of the carbohydrate sources in the four whole diets, which are modified corn, pea, lentil and faba bean, and compare these to glycemic responses in dogs and cats. I determined if digestibility is related to glycemic response which is why next I compared the glycemic, insulinemic and cardiovascular responses to an oral glucose tolerance test and a single feeding of the whole diets following six weeks of diet consumption for evaluation of long-term health effects. I also determined postprandial levels of MG and nitrotyrosine as a measure of metabolic health and oxidative stress.

1.4. Hypotheses

The main hypothesis of this thesis work was that pulse starches will have low glycemic index values and therefore produce diets with reduced glycemic and insulinemic responses which will provide long-term health benefits in dogs and cats. Specifically, the hypotheses for each study are:

Study 1: Acute Postprandial Responses to Starches and Whole Diets in Dogs and Cats

1) *In vitro* digestibility of pulse starches will be low compared to commonly used starch sources such as unmodified corn, modified corn, rice flour, white wheat flour, tapioca, and potato with *in vitro* digestibility correlating with glycemic responses in both species.
2) After carbohydrate consumption, cats will have greater postprandial increases in plasma glucose, insulin, methylglyoxal and adverse cardiovascular changes compared to dogs.

3) Pulse starches and diets formulated with 30% inclusion of pulse starches will produce lower peak plasma levels and AUC for postprandial glucose and insulin compared to commonly used pet food ingredients such as corn, rice and wheat.
4) Higher glycemic index starches and diets will produce higher postprandial increases in plasma methylglyoxal and greater adverse cardiovascular changes compared to low glycemic index starches or diets. These increases will be higher in cats than dogs and lead to greater adverse effects.
5) Extrusion of whole diets will increase the glycemic index value and adverse postprandial cardiovascular and metabolic responses.

Study 2: Long-term Health Benefits of Pulse Verses Corn-Based Formulated Diets

1) Pulse starch diets (pea, lentil and faba bean) will have lower *in vivo* digestibility than the modified cornstarch diet in both dogs and cats, but *in vivo* digestibility will be comparable between species.
2) Following six weeks of feeding in both species, the responses will not change from those of the animals not previously acclimated to the test diets and that the lower digestibility of pulse starches will produce lower postprandial increases in MG, oxidative stress and negative cardiovascular effects compared to modified cornstarch in both species.
3) Following six weeks of feeding in both species, pulse starch-containing diets, as compared to the modified cornstarch diet, will result in better glucose tolerance, insulin sensitivity and overall metabolic and cardiovascular health benefits after challenge with either glucose or the test diet.
4) Beneficial effects of long-term feeding of diets formulated with 30% pulse starches compared to modified cornstarch will be more pronounced in cats compared to dogs.

2. LITERATURE REVIEW

2.1. Overview

The health of companion animals such as dogs and cats is now recognized as an important part of good human mental and physical health (Peacock et al. 2012; Shubert, 2012). Some studies even report a link between owner and pet obesity (Shearer. 2010), which is now being seen as a One Health problem (Sandoe et al. 2014). Owners are becoming increasingly aware of pet nutrition and are willing to pay more for their pet's nutrition and medical care. Therefore, pet food companies are constantly changing their products to better meet the nutritional and energy needs for our pets and prolong their lives. The energy in pet foods comes mainly from carbohydrate sources, since protein is the most expensive component used in pet formulations. Grain-free products are becoming increasingly viewed as a healthier alternative to grain-based food, with many pet food companies switching their formulations to meet the demands (Laflamme et al. 2014), despite a lack of evidence that grain-free foods are healthier for pets than foods with grains. Therefore, the use of pulse starches as a carbohydrate source in pet foods would be economically valuable, particularly for Saskatchewan-grown pulses, and may show overall better health benefits in companion animals.

2.2. Carnivores vs Omnivores

Domestic cats (*Felis catus)* and dogs (*Canis familiaris),* although fed surprisingly similar pet food diets, have different nutritional needs. Cats are strict carnivores and have evolved to consume a diet with higher proportions of protein and fat with very little carbohydrate, whereas dogs are carno-omnivorous animals that can consume a more diverse diet (Batchelor et al. 2011; Bosch et al. 2015). Cats require specific additional nutrients such as arachidonic acid and taurine which they need to obtain from their carnivorous diet (MacDonald et al. 1984; Thompson, 2008)

whereas dogs can obtain these nutrients such as taurine and arginine by other means (Bosch et al. 2015). Despite these differences, these species do share similarities such as short digestive tracts and lack of salivary amylase (National Research Council, 2006). Domestication of cats has led to a change in diet from prey they would catch in the wild, such as small rodents and birds, to a commercial food that can contain up to 50% carbohydrate of which is commonly corn, rice or wheat. Consumption of small prey in the wild leads to less than 4% of metabolizable energy from carbohydrates (Eisert. 2011). Recently it has been recognized that there are high incidences of feline obesity, insulin resistance and type 2 diabetes, which may be due to domestication and a more sedentary lifestyle (Rand et al. 2004; Slingerland et al. 2009; Verbrugghe et al. 2012). Feline diabetes closely resembles human type 2 diabetes and similar to humans, is associated with obesity (Hoenig. 2012). Through domestication, dogs have become able to more efficiently utilize higher amounts of carbohydrates in their diet, because they are carno-omnivorous species (Arendt et al. 2014). Even though their carbohydrate utilization is higher, some studies indicate they should not be consuming as much carbohydrates as found in most commercial dog foods (Farrow et al. 2013). Obesity in dogs is becoming increasingly common, with 30-60% being overweight or obese (Bland et al. 2010; Courcier et al. 2010), while Lund et al. (2005 & 2006) reports that one in three dogs and cats are overweight. Dogs also suffer from insulin resistance which can be induced by obesity and improved with weight loss (German et al. 2009). Overweight and obese states in dogs and cats are determined using a body condition scoring system, with the most commonly used system developed and validated at Purina (Table 2.1; Laflamme. 1997a, 1997b).

In the intestine, carbohydrates are broken down into monosaccharides by pancreatic enzymes, and brush border hydrolases to form glucose, galactose and fructose (Figure 2.1;

Hediger and Rhoads. 1994). Glucose is transported across the brush border membrane of the intestine by sodium/glucose cotransporter-1 (SGLT1), which is considered the rate limiting step for entry of glucose across the intestine into the blood (Batchelor et al. 2011). Although carnivores express SGLT1, it has been suggested that they are unable to upregulate intestinal glucose transport when fed food high in carbohydrates and that carnivores may absorb glucose via a non-sodium dependent pathway (Batchelor et al. 2011). In contrast, this transporter has been reported to be approximately 2-fold higher in the intestine of dogs compared to cats, with dogs having the ability to upregulate SGLT1 (Batchelor et al. 2011). Cats also have very low levels of amylase, the enzyme responsible for the start of starch digestion, from the salivary glands and pancreas (Kienzle. 1993a; Batchelor et al. 2011). Two enzymes responsible for glucose metabolism are hexokinase and glucokinase. Hexokinase activity is significantly higher in the feline liver than the canine liver, while glucokinase activity and gene expression has been reported to be minimal or absent in the cat liver (Washizu et al. 1999; Tanaka et al. 2005). It has been suggested that the function of the feline liver is instead to produce glucose via gluconeogenesis rather than to remove glucose from the blood (Tanaka et al. 2005; Verbrugghe et al. 2012). Carnivorous species are reported to be in a constant state of gluconeogenesis, similar to ruminants (MacDonald et al. 1984), which is slightly increased postprandial (Case et al. 2011). This is opposite compared to omnivores that show maximal gluconeogenic rates during fasting (MacDonald et al. 1984). Compared to the dog, these differences in metabolism may reduce the cat's ability to decrease the hyperglycemia that can occur after feeding a meal containing high amounts of starch or glucose, consistent with that reported by Farrow et al. (2012). Based on this limited information, I posed the question: will there be further differences between cats and dogs in glucose handling?

Table 2.1 Body condition score system for dogs and cats

Score	Description	
	DOG	*CAT*
1	Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss of muscle mass.	Ribs visible on shorthaired cats. No palpable fat. Severe abdominal tuck. Lumbar vertebrae and wings of ilia easily palpated
2	Ribs, Lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominences. Minimal loss of muscle mass.	Ribs easily visible on shorthaired cats. Lumbar vertebrae obvious. Pronounced abdominal tuck. No palpable fat.
3	Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist and abdominal tuck.	Ribs easily palpable with minimal fat covering. Lumbar vertebrae obvious. Obvious waist behind ribs. Minimal abdominal fat.
4	Ribs easily palpable, with minimal fat covering. Waist easily noted, viewed from above. Abdominal tuck evident.	Ribs palpable with minimal fat covering. Noticeable waist behind ribs. Slight abdominal tuck. Abdominal fat pad present.
5	Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked up when viewed from side.	Well-proportioned. Observe waist behind ribs. Ribs palpable with slight fat covering. Abdominal fat pad minimal.
6	Ribs palpable with slight excess fat covering. Waist is discernible viewed from above but is not prominent. Abdominal tuck apparent.	Ribs palpable with slight excess fat covering. Waist and abdominal fat pad distinguishable but not obvious. Abdominal tuck absent.
7	Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and base of tail. Waist absent or barely visible. Abdominal tuck may be present.	Ribs not easily palpated with moderate fat covering. Waist poorly discernible. Obvious rounding of abdomen. Moderate abdominal fat pad.
8	Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck. Obvious abdominal distention may be present.	Ribs not palpable with excess fat covering. Waist absent. Obvious rounding of abdomen with prominent abdominal fat pad. Fat deposits present over lumbar area.
9	Massive fat deposits over thorax, spine and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distention.	Ribs not palpable under heavy fat cover. Heavy fat deposits over lumbar area, face and limbs. Distention of abdomen with no waist. Extensive abdominal fat deposits.

(adapted from Laflamme, D.P. 1997a; 1997b)

2.3. Carbohydrates

Carbohydrates are one of the main sources of energy for cells (Guyton & Hall. 1997). They are composed of mono-, di- and polysaccharides, which are broken down (if not already monosaccharides) to form glucose, fructose and galactose during digestion. The breakdown of carbohydrates into glucose, and the transport of glucose through the body is shown in Figure 2.1. Carbohydrates make up between 60-90% of the dry-matter weight of plants and are the main energy-containing components (Case et al. 2011). They are also a main component of pet foods making up between 30-60% of dry foods and up to 30% of canned foods currently on the market, which in the case of cats, can be up to 20 times the normal metabolizable energy (ME) density of the food that they would consume in the wild (Eisert. 2011).

Starch is a non-structural plant polysaccharide (Case et al. 2011) and the major carbohydrate of pulses, accounting for 22-45% of the dry matter weight (Hoover et al. 2010). In both dogs and humans, carbohydrates are the main nutrient responsible for changing post-prandial blood glucose and therefore insulin levels as well (Carciofi et al. 2008). The same may not be true for cats (de-Oliveira et al. 2008; Verbrugghe et al. 2010), although this is still an area of debate. In non-diabetic humans, greater postprandial glucose responses are linked to starches that are more rapidly and completely digested/absorbed and have a higher GI value (Wolever & Bolognesi. 1996). Glycemic index is calculated by comparing the time-course of postprandial glucose responses from a reference or standard food source, usually pure glucose or white bread, to the glucose responses from a test food (Figure 2.2).

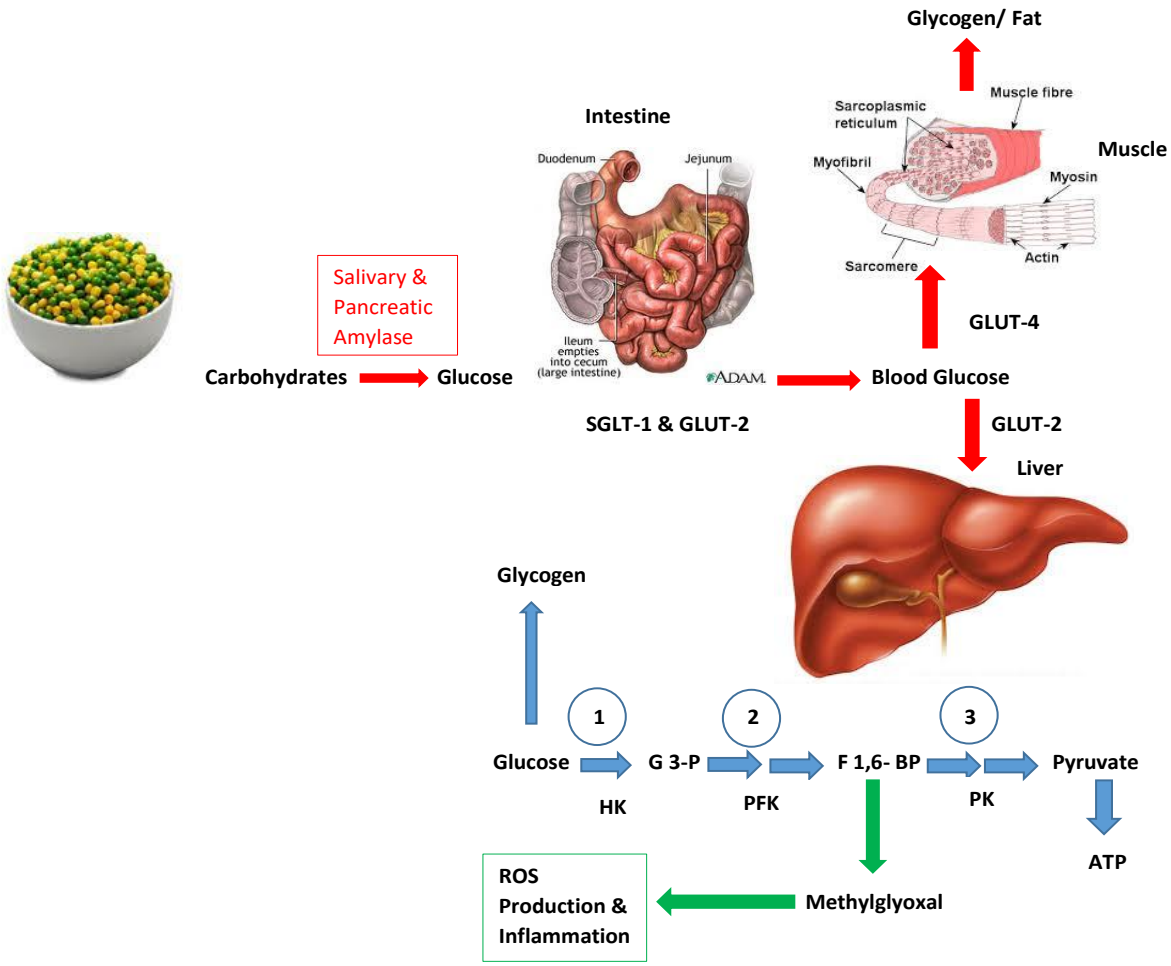
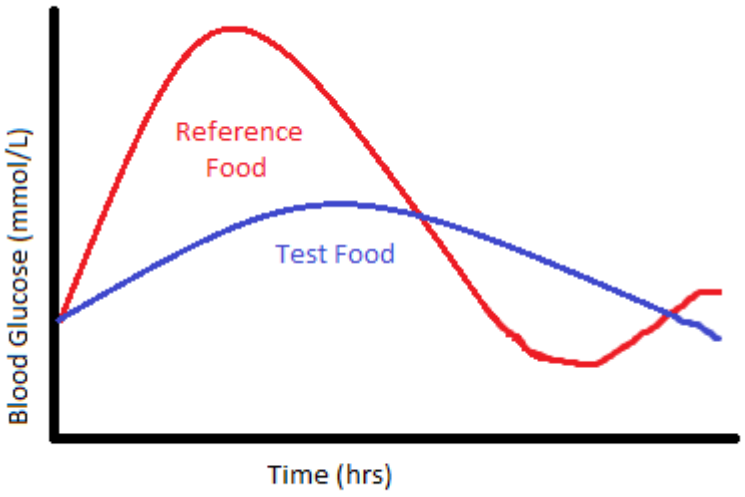


Figure 2.1 This figure shows the breakdown of carbohydrates into ATP, or storage as glycogen or fat. The red arrows indicate transport of glucose from the intestine into the blood then into the liver or muscle where there is insulin dependent glucose disposal. The blue arrows indicate the breakdown of glucose inside the liver. The green arrows indicate that some glucose may be shunted via F 1,6-BP to produce reactive glucose metabolites. SGLT-1 = sodium glucose transporter-1; GLUT-2 = glucose transporter; GLUT-4 = insulin dependent glucose transporter; G 3-P = glucose 3-phosphate; F 1,6-BP = fructose 1,6-bisphosphate; 1 (HK) = hexokinase; 2 (PFK) = phospho-fructokinase; 3 (PK) = pyruvate kinase. 1-3 are all rate limiting steps in glycolysis.



$$Glycemic\ Index\ (GI) = \frac{\text{Glycemic AUC to test food}}{Glycemic\ AUC\ to\ reference\ food}\ x\ 100$$

Figure 2.2 Calculation and example of a blood glucose curve for calculating glycemic index. AUC = glucose time course area under the curve.

In comparison, cats and dogs fed similar high starch diets took a relatively longer time to reach maximum blood glucose levels than humans. De-Oliveira et al. (2008) have shown a significant increase in blood glucose at both four and 10 hours postprandial in cats when fed whole diet meals containing cornstarch. Hewson-Hughes et al. 2011 reported that following consumption of a high starch diet (31.7g/100g whole corn) dog blood glucose levels to return to baseline between 3-5 hours postprandial, where as levels were still significantly elevated 19 hours postprandial in cats.

Similar findings were reported by Farrow et al. 2013 in cats following consumption of a diet containing 50% ME from carbohydrates (corn and sorghum). This relatively slower glycemic response may be caused by their lower activity of starch digesting enzymes, such as amylase, in cats compared to dogs (Hewson-Hughes et al. 2011). Moreover, cats achieved significantly higher peak blood glucose than the pre-meal concentrations compared to dogs (Hewson-Hughes et al. 2011). Not only are cats thought to utilize ingested glucose poorly, but continued gluconeogenesis may make them quite different than dogs (Legrand-Defretin. 1994). However, it must be cautioned that findings in all of these previous studies in cats and dogs are confounded by the fact that proper GI testing methodology was not followed. Methodological deficiencies include animals being fed whole diets containing protein and/or fat plus a failure to limit food consumption to the beginning of the test. This makes determining species differences in glycemic response to just carbohydrates impossible to this point until proper experiments are conducted.

The major components of starch are amylose and amylopectin (Hoover et al. 2010). The ratio of these two components influence the digestibility of the starch and relative content varies

among starch sources. Amylose, a linear molecule, contains mostly α(1→4) linkages and is less susceptible to digestion, whereas amylopectin, a branched molecule, contains chains of α(1→4)-D-glucose residues connected by α(1→6) linkages that digesting enzymes can act on more readily (Ratnayake et al. 2002). Starches can be classified into three classes: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), which are determined by in vitro starch digestion rates (Englyst et al. 1992; Singh et al. 2010). It is thought that high in vitro and in vivo starch digestibility corresponds to high glycemic response (Hoover et al. 2010), but this is an area of great scientific debate. Furthermore, Sun et al. (2006) reports that they were not able to establish a relationship between in vitro and in vivo ileal digestibility of pea, barley, and potato starches in pigs, and that the in vitro was higher than the in vivo. RDS is absorbed quickly and is therefore thought to cause a faster, higher rise in postprandial blood glucose. In contrast SDS is digested at a slower rate than RDS in the small intestine. RS is thought to lack contribution to the glycemic response and may instead be fermented in the large intestine (Englyst et al. 1992; Hoover et al. 2010). SDS tends to have a higher ratio of amylose compared to amylopectin, making it more slowly digestible compared to RDS. RS has an even higher amount of amylose compared to SDS (Wang & Copeland. 2013). Consuming diets high in SDS may contribute to better control of blood glucose levels, and therefore management of diabetes (Lehmann & Robin. 2007). Cornstarch is reported to be 99% digestible in humans, whereas legume starches are lower (80-90%) (Singh et al. 2010). Therefore, I hypothesized that the lower digestibility of pulse starches would translate into lower blood glucose and insulin responses, compared to cornstarch in both cats and dogs

2.3.1. Effects of Processing

The properties of starches can be changed with different processing techniques such as extrusion and purification which impact their susceptibility to enzymatic breakdown. When starches are heated in excess water, they go through a process known as gelatinization, where the starch granules absorb water. Gelatinization irreversibly disrupts the hydrogen bonds and ordered structure of starch (Wang & Copeland, 2013). Erayu et al. (2009) showed that the level of RDS increases while the level of RS decreases in peas and beans when boiled or pressure cooked. Increasing the amount of RDS should then increase the rate of digestibility, assumedly making for a faster, higher glycemic response but this has yet to be definitively demonstrated *in vivo*. The opposite, known as retrogradation, can happen upon cooling or desiccation. The starch granules are able to realign into a more ordered structure (Wang & Copeland, 2013), which may make them more resistant to digestion. Eerlingen et al. (1994) reported a lower rate of starch enzymatic breakdown, suggesting that retrogradation could lower the glycemic response, but again this has not been demonstrated *in vivo*. Extrusion is a process that uses high temperature and pressure to form a pelleted, dry form of food (Berrios. 2013). This process can cause partial gelatinization of the starch during the extrusion phase (Parada et al. 2011) which can increase the digestibility of the food and therefore increase the glycemic response. However, depending on how rapidly the hot pellet is cooled, retrogradation may also occur. Thus, not only must the starch source be considered, but so too must extrusion conditions in producing an animal food. Formulation of a whole diet will also influence the digestibility of the starch and therefore the GI. Fat added to the whole diet will delay gastric emptying (Guyton & Hall. 1997), which should decrease the GI as reported by Henry et al. (2008). Many carbohydrate sources include fibre (soluble and/or insoluble), which has been reported to reduce energy digestibility, glucose

responses (Fischer et al. 2012) and reduce gastric emptying (Howarth et al. 2001), thereybmodifying carbohydrate metabolism and GI.

Chemical or enzymatic modifications through processing is another way to change the function and digestion rates of starches (Chung et al. 2008b). For this thesis I evaluated the difference modification to cornstarch, in particular hydroxypropyl cross linking, has on *in vitro* digestibility and *in vivo* glycemic and insulinemic responses. Hydroxypropyl modifications have been shown to decrease *in vitro* digestibility of pea starch (Hoover et al. 1988) and of cornstarch (Chung et al. 2008b) which also lowered the estimated GI. Estimated GI is an *in vitro* estimation of the glycemic response which uses the % starch hydrolysis as a surrogate for *in vivo* testing (Goni et al. 1997). Based off this information, I determined if modified cornstarch would have a lower *in vitro* digestibility than unmodified cornstarch and if this would translate into a low *in vivo* digestibility comparable to pulse starches, with correspondingly lower glycemic and insulinemic responses compared to unmodified cornstarch in both dogs and cats.

2.3.2. Pulses and Glycemic Index (GI)

Glycemic index refers to a ranking system that is based on the starch digestion of foods and their effects on blood glucose in humans compared to a standard source such as white bread or glucose. By definition, the standard source has a GI of 100. Lentils have been shown to have a GI around 40 when compared to white bread in humans, although values as low as 29 have been reported (Jenkins et al. 1981). In fact, most pulses have GI values in the range of 30-60 in humans depending on the form of pulse, such as whole pulses or pulse flour (Foster-Powell et al. 1995; Foster-Powell et al. 2002). Consumption of low GI carbohydrates, such as pulses, has been reviewed and linked to various health benefits in terms of cardiovascular disease risk, diabetes mellitus, aging and reduced oxidative stress in humans (Mudryj et al. 2014). In contrast, corn has

a high GI value in the range of 90-100 in humans. This is likely due to the much higher starch digestibility which is determined by the amount of amylose and amylopectin in the starch source. Although GI has become an accepted measure of glycemic response in humans, this is not so in cats and dogs. Moreover, the methodology is not validated in these species. There is virtually no work documenting GI values for common starch sources in pets (Aldrich. 2013). Previous work from this laboratory has shown that peas have a low GI in healthy dogs compared to barley, corn and rice (Adolphe et al. 2012). This low GI of peas also produced the lowest insulin response compared to other carbohydrate sources in dogs (Adolphe et al. 2012). When peas are formulated into whole diets the GI increased due to formulation and processing effects, but was still lower than a diet formulated with rice (Adolphe et al. 2015). Mori et al. (2016) stated that cornstarch added to a diet produced a high GI in cats, but no actual values were calculated or shown in the results. Carcifio et al. (2008) also reported that pulse-based diets may play a role in reducing the change in blood glucose and insulin concentrations by extending the time over which glucose is absorbed in dogs. Therefore, more work is needed to determine GI values for a broad range of pulses in dogs, but especially in cats where no GI values have been reported. This is necessary information to support the claim that pet food containing pulses are low GI and provide health benefits for pets.

Canadian grown pulses are an important sector of the agricultural industry which has been growing rapidly over recent years, and as of 2010 was valued at over one billion dollars (Hoover et al. 2010). Saskatchewan is the main centre of the Canadian pulse industry, growing 96% of Canada's lentils, and 70% of Canada's dry pea crop in 2012 (Saskatchewan Pulse Growers. 2017). Canada is also the leading exporter of peas, lentils and chickpeas with numerous international markets such as India, U.S.A, China, and Turkey (Saskatchewan Pulse Growers.

2017). Therefore, if pulses are determined to be low GI and produce healthier glycemic responses in both cats and dogs, this could be used in marketing Saskatchewan grown pulses for inclusion in pet foods and further increase sales of these crops.

2.4. Glucose and Insulin

Glucose is formed when carbohydrates are broken down into useable energy for cells (Guyton & Hall. 1997) which then travels in the blood stream and passes through the liver. Excess glucose in the blood can either be taken up by the liver and stored as glycogen, or sequestered into other glucose-disposing organs such as skeletal muscle in an insulin-dependent manner. This prevents excessive hyperglycemia (MacLean. 1924). Glycogen can be converted back to glucose in response to energy requirements of the body so that ideal blood glucose levels are maintained and glucose is available for use by tissues at any given time. Chronic hyperglycemia (or diabetes) has been linked to many negative health effects including the pathogenesis of vascular dysfunction (Di Carli et al. 2003). An early indicator of vascular pathology is thought to be impairment of endothelial-dependent vasodilation through production of reactive oxygen species (ROS) (Kawano et al. 1999). Prolonged endothelial dysfunction and arterial stiffening are known to lead to atherosclerosis (Anderson. 1997), hypertension, cardiovascular disease and death from heart failure in humans (Marti et al. 2012).

Insulin is a peptide hormone, produced in the beta cells of the pancreas, which is important for regulating carbohydrate and fat metabolism in the body (Guyton & Hall. 1997). Insulin is released from the pancreatic beta cells in response to increased levels of blood glucose in humans. Cells become highly permeable to glucose once insulin has bound to specific membrane receptors (Guyton & Hall. 1997). Normal fasting plasma insulin levels in humans are in the range of 30-60 pmol/L (Guyton & Hall. 1997), and in dogs it is reported to be 4.2 ± 0.7 pmol/L

(Adolphe et al. 2012). Baseline values for cats were reported by de-Oliveira et al. (2008) to be in the range of 3.3-3.9 mmol/L for glucose and between 0-30 pmol/L for insulin. Peak insulin levels were reported to occur at 30 minutes for humans with levels of 567 pmol/L (Reaven & Miller. 1968), following an oral glucose load. Peak insulin levels in beagle dogs were reported to also occur at 30 minutes following an oral glucose load, with levels of 458 pmol/L (Adolphe et al. 2012). Peak insulin levels for lean cats have similarly been reported to reach maximum levels at 30 minutes post-feeding in a glucose tolerance test (Hoenig et al. 2010). Following a single feeding of different starch sources in cats, maximal insulin levels have been shown to range from 180-200 pmol/L when fed corn or sorghum, and 145 pmol/L when fed lentils (de-Oliveira et al. 2008), although these tests were performed using whole diet meals that included fat and protein along with the starch sources.

Hewson-Hughes et al. (2011) showed that when cats are fed high starch diets, plasma insulin levels were significantly elevated from 3-7 and 11-17 hours after feeding. The type of starch can also affect the insulin response, although for cats this may not be the main determinant of postprandial insulin responses (Verbrugghe et al. 2010). Work done by de-Oliveira et al. (2008) shows in cats a higher postprandial peak and shorter time to peak in insulin when fed a cornstarch-based diet compared to a pea or lentil starch-based diets. Previous work done in this laboratory reported that the peak insulin response in dogs was greatest after feeding a glucose solution compared to peas, corn, rice or barley, but that there were no significant differences in the AUC for insulin response among these complex carbohydrate sources (Adolphe et al. 2012). Carciofi et al. (2008) reported, in dogs, higher mean insulin concentrations following a corn diet compared to a lentil diet, but that the postprandial insulin peak and AUC were not significantly different among corn, rice, pea or lentil diets. In a long-term feeding study (Adolphe et al. 2015),

insulin sensitivity improved following a glucose challenge in the dogs fed a pea diet compared to the rice diet. Therefore, I hypothesized that I would see species differences between dogs and cats in insulin responses to both the corn and pulse diets, and that the benefits of pulses would be more pronounced in cats compared to dogs.

Diabetes mellitus is a metabolic disease in which a person or animal has prolonged hyperglycemia, either because the pancreas does not produce enough insulin or because cells do not respond to the insulin produced (Canadian Diabetes Association. 2013). Blood glucose parameters have been set to determine whether a human is normal, prediabetic or diabetic (see Table 2.2). For dogs and cats, there is a lack of established guidelines for determination of prediabetes and diabetes mellitus is usually only diagnosed when animals present with chronic hyperglycemia (Nelson & Reusch. 2014). Type 1 diabetes is defined as a condition when the pancreas does not produce enough insulin or any insulin at all, so insulin must be given as an injection or insulin pump. In contrast, type 2 diabetes is defined as cells failing to respond sufficiently to insulin, to lower blood glucose, also referred to as insulin resistance. Obesity in humans has been clearly linked to type 2 diabetes mellitus and insulin resistance (Mokdad et al. 2003), but this may also be true in cats and dogs (German et al. 2010). Insulin resistance resulting from chronic feeding of a high glucose diet can lead to obesity, type 2 diabetes, and cardiovascular disease in humans (Reaven. 2011). Type 2 diabetes accounts for over 80% of reported cases of diabetes in both cats (Rand et al. 2004; Case et al. 2011; Nelson & Reusch. 2014) and humans (Canadian Diabetes Association. 2013; Verkest. 2013), whereas dogs are more likely to exhibit type 1 diabetes (Rand et al. 2004; Case et al. 2011; Nelson & Reusch. 2014). Despite this, dog obesity is associated with insulin resistance (Larson et al. 2003; Verkest. 2013).

Table 2.2 Fasting and postprandial blood glucose levels for normal healthy humans, dogs and cats, individuals and animals with risk for diabetes or prediabetes, and individuals and animals with diagnosed diabetes.

	Humans	Dogs	Cats
FASTING			
Normal	3.9-5.5 mmol/L	< 6.7 mmol/L	< 6.7 mmol/L
Prediabetic	5.6- 6.9 mmol/L	6.7-10 mmol/L	6.7-12 mmol/L
Diabetic	≥ 7 mmol/L	10- ≥ 12 mmol/L	12- ≥ 16 mmol/L
POST PRANDIAL			
Normal	< 7.8 mmol/L	4.3-11.1 mmol/L	3.5-13.8 mmol/L
Prediabetic	7.8-11 mmol/L		
Diabetic	≥ 11.1 mmol/L	≥11.1 mmol/L	≥16.7 mmol/L

Values for humans obtained from the Canadian Diabetes Association and the American Diabetes Association. Dog & Cat values obtained from: Canine and Feline Endocrinology, 4th edition. 2015; Adolphe et al. 2012; Theiss et al. 2004; Elliott et al. 2012;

The same association has been shown in cats (Hoenig et al. 2011) in which a 1 kg increase in body weight leads to a 30% decrease of insulin sensitivity (Hoenig et al. 2007). Chronic intake of high levels of carbohydrates has been proposed to induce feline insulin resistance, whereas lowering levels of carbohydrates may improve insulin sensitivity in non-obese cats (Farrow et al. 2002). However direct evidence for this hypothesis in cats or dogs is sparse. It has even been suggested that both high and low carbohydrate contents can negatively affect insulin sensitivity in cats (Verbrugghe et al. 2010). Although some cats appear to be predisposed to developing glucose intolerance because of naturally occurring low insulin sensitivity (Case et al. 2011), the type of carbohydrate may also influence the tendency to develop insulin resistance. I hypothesized that using low GI starches would improve insulin sensitivity compared to food prepared with high GI starch sources in both cats and dogs, but that cats would show the greatest benefit.

2.5. Methylglyoxal

Methylglyoxal (MG) is formed in all cells and organisms (Thornalley, 1996) and is a highly reactive dicarbonyl compound. MG is formed endogenously through a non-enzymatic process mainly through side reactions (see Figure 2.1) (Dhar et al. 2008). MG is formed by the elimination of phosphate from the phospho-ene-diolate form of gyceraldehyde-3-phosphate and dihydroxyacetonephosphate (DHAP) (Thornalley. 1996). MG can be produced from numerous precursors, such as D-glucose, fructose and aminoacetone, all of which have shown to induce concentration-dependent increases in MG (Dhar et al. 2008). MG is also formed exogenously in numerous foodstuffs during processing and fermentation (Ankrah et al. 1999). MG is a highly toxic molecule that interacts with arginine and lysine residues in many different proteins to yield

irreversible advanced glycation end products (AGEs) (Jia et al. 2006). Elevated MG also increases oxidative stress (Desai et al. 2010) and inflammation (Dhar et al. 2008). AGEs are associated with cardiovascular complications of diabetes, age-related neurodegenerative disease, and connective tissue disorders (Sena et al. 2012). Consumption of a high AGE diet in rats has been shown to increase RAGE, the pro-inflammatory receptor for AGEs (Poulsen et al. 2016). Elevated MG has also been reported to play a key role in the pathogenesis of insulin-resistance (Jia et al. 2006) and has been shown to induce abnormalities characteristic of type 2 diabetes in rats (Dhar et al. 2011) as well as increase blood pressure by increasing renin-angiotensin levels in rats (Dhar et al. 2014). Physiological concentrations of plasma MG have been reported to range from 0.2- 14.2 μM in rats, and around 1.4 μM in healthy humans (Jia et al. 2006).

The degradation and detoxification of MG mainly happens via the glyoxalase system (Sousa Silva et al. 2013), which consists of two enzymes that both require reduced glutathione (GSH) (Kalapos, 1999). Under physiological conditions, MG forms D-lactate via the glyoxylase system and produces reduced glutathione (Sena et al. 2012). As shown in mice, the GSH content in blood was significantly reduced after exposure to MG, compared to the controls, thereby interfering with other GSH dependent functions (Ankrah et al. 1999). GSH is critically important for detoxifying ROS, with decreased GSH leading to increased oxidative stress and subsequent tissue damage including vascular dysfunction. Both oxidative stress and MG have the ability to deplete GSH which can cause positive feedback and lead to increased levels of both with subsequent negative effects.

In previous studies from this laboratory, MG levels were elevated in normal beagle dogs after a single glucose feeding, but not after feeding complex carbohydrate sources (peas, barley, corn and rice) (Adolphe et al. 2012). This was the first evidence of postprandial increase in MG levels

in any non-human species. Subsequently postprandial increases in MG production were reported in healthy humans following an oral glucose test (Masterjohn et al. 2012). Glucose tolerance has been shown to decrease in rodents following chronic MG administration, suggesting that chronic excessive postprandial MG production may produce glucose intolerance in otherwise healthy animals (Ankrah et al. 1999). MG levels were significantly increased in the serum and aorta of Sprague Dawley rats when chronically administered fructose (Wang et al. 2008). This same study also found the thickness of the walls of the mesenteric arteries increased, and arterial stiffening leading to increases in blood pressure. MG has also been linked to obesity. Studies have shown that MG levels correlate with serum LDL and triglyceride concentrations (Turk et al. 2011) in diabetic patients and that MG induces changes to adipose tissue which can lead to obesity (Matafome et al. 2012).

A two to four-fold increase in blood plasma MG levels have been reported in humans with both Type 1 and 2 diabetes mellitus (Wang et al. 2007). MG has also been associated with endothelial dysfunction induced by diabetes in humans (Sena et al. 2012). MG also covalently binds to the internal arginine in the B-chain of insulin, which significantly reduces its capacity to stimulate glucose uptake and could be the link between hyperglycemia and insulin resistance (Jia et al. 2006). Furthermore, MG has been shown to contribute to the pathogenesis of type 2 diabetes by having damaging effects on INS-1E cells, leading to adduct formation and the impairment of both the secretion and action of insulin by pancreatic beta-cells (Fiory et al. 2011). Long-term exposure of rat mesenteric artery to MG reduced acetylcholine dependent endothelium relaxation (Mukhoda et al. 2013) whereas the same group showed that short term exposure did not which could be a link between diabetic associated cardiovascular disease. With this information I hypothesized that MG levels would be increased in both cats and dogs when

fed a standard glucose source, or corn, as compared to pulse starches, but that cats would experience higher postprandial MG levels. As a result of increased MG production and subsequent toxicity, I hypothesized that cats would suffer greater adverse effects compared to dogs, which would be more evident in the long-term study than the short term.

2.6. Oxidative Stress

Oxidative stress is caused by an excess amount of free radicals or reactive oxygen species (ROS) in the body. Free radicals are highly reactive atoms or molecules which have an unpaired orbiting electron, such as the superoxide anion ($O_2^{-\cdot}$) and hydroxyl radical ($OH^{\cdot}$) (Kojda & Harrison, 1999). Free radicals are able to readily covalently bind to other molecules, such as proteins and DNA, thereby altering their structure and function. Oxidant stress is thought to affect several important functions in the vascular wall such as regulation of blood flow, inhibition of platelet aggregation and control of cellular growth (Kojda & Harrison, 1999). Oxidative stress has also been implicated in physiological responses to aging and exercise, as well as pathological conditions such as cancer, neurodegenerative disease (Nunomura et al. 2001), cardiovascular disease, diabetes, and toxicity (Preiser, 2012). Moreover, it has been shown that biomarkers of oxidative stress are elevated in the pancreatic islets of type 2 diabetic humans (Del Guerra et al. 2005). Increases in the oxidative stress biomarker, catalase, has been reported in diabetic dogs as compared to healthy dogs (Chansaisakorn et al. 2009). Also, decreases in antioxidant levels and superoxide dismutase have been reported in diabetic cats compared to healthy cats (Webb & Falkowski, 2009).

For this thesis, a nitrotyrosine enzyme-linked immunosorbent assay (ELISA) was used as an indicator of oxidative stress. Nitrotyrosine is indirect evidence of prior peroxynitrite production ($ONOO^{-\cdot}$) (Ceriello. 2002). An increase in ONOO-$^{\cdot}$ results from a reaction between nitric oxide

($NO^{\cdot}$) and superoxide ($O_2^{-\cdot}$). Once formed, $ONOO^{-\cdot}$ stably reacts with tyrosine residues, as well as other macro molecules and thus nitrotyrosine can be used as an index of $ONOO^{-\cdot}$ - production. Ceriello (2002) reported that elevated nitrotyrosine has been detected in the plasma of diabetic patients, but not in healthy non-diabetic humans. In contrast, Wang et al. (2004) reported that nitrotyrosine levels were detected in both diabetic and healthy humans, with no significant differences between the two. Even in normal humans, nitrotyrosine levels will become elevated when exposed to a continuous infusion of glucose (Ceriello, 2002). Therefore, I wanted to determine if I would be able to detect nitrotyrosine levels in plasma from healthy dogs and cats, then determine if this changed with diet.

$NO^{\cdot}$ is an important but unstable (Kojda & Harrison. 1999) endothelium-dependent vasodilator and is responsible for vascular smooth muscle relaxation (Ignarro et al. 1987). Mammals synthesize $NO^{\cdot}$ from L-arginine by nitric oxide synthases, of which there are several forms (Russo et al. 2002) including endothelial, neuronal and inducible isoforms (Russo et al. 2002; Marti et al. 2012). One indicator of endothelium dysfunction is decreased levels of $NO^{\cdot}$ which can be caused by dysfunctional synthesis or by inactivation of $NO^{\cdot}$ from enhanced formation of ROS (Russo et al. 2002). There is good evidence that in the vascular wall the bioavailability of $NO^{\cdot}$ is decreased because of excessive $O_2^{-\cdot}$, which has been found in such conditions as atherosclerosis, hypertension, and diabetes mellitus (Kojda & Harrison. 1999). $NO^{\cdot}$ activity can be increased with the addition of glutathione, to help reverse endothelial dysfunction (Prasad et al. 1999

2.7. Cardiovascular Parameters

2.7.1. Heart Rate and Blood Pressure

Because of this possible link between postprandial hyperglycemia to MG, ROS, endothelial function, inflammation and their associated risk for cardiovascular impairment, I measured the blood pressure of the cats and dogs by high definition oscillometry (HDO). Normal canine blood pressures are reported to differ amongst breeds, with larger breed dogs tending to have lower overall blood pressure, compared to small or toy breeds (Egner et al. 2003). From previous data collected in this laboratory, the average blood pressure for beagle dogs using an HDO system is 129/67 mmHg, with an average pulse rate of 84 ± 18 (Adolphe et al. 2015), which agrees with the range reported by Egner et al. (2003). The average blood pressure values for cats do not appear to be breed-specific, and the normal feline blood pressure has been reported to be 124/84 mmHg (Egner et al. 2003). Changes in heart rate (HR) and blood pressure (BP) are reported to increase in normal beagle dogs (Miyazaki et al. 2002) and cats (Matsukawa & Ninomiya. 1987) during a feeding period, but then decrease during the postprandial period (Miyazaki et al. 2002), although other studies report no association between feeding and HR or BP changes in dogs (Piccione et al. 2005). In humans, postprandial increases in HR and systolic pressure, and decreases in diastolic pressure have been reported when fed a standard meal (Mey et al. 1993). Cardiovascular changes are also associated with health status, with Usha-Rani et al. (2013) showing that both pre and postprandial BP and HR are increased in obese women. Similarly, in dogs, Adolphe et al. (2015) reported increased HR but not BP after 12 weeks of obesity. For the acute study I hypothesized that I would not see postprandial cardiovascular changes in either species but for the long-term study I hypothesized that I would see higher pre- and postprandial blood pressure and heart rate in the cats and dogs following a feeding of high GI starch diet, compared to low GI pulse starch diet.

2.7.2. Arterial Ultrasound

Doppler ultrasound allows evaluation of blood flow direction and velocity, whereas the two- and three-dimensional ultrasound imaging modes (B-mode) allows visualization of structure and diameter of the artery (Nyland & Matooon. 2002). The median artery will be visualized in the dogs to evaluate endothelial function and post prandial effects. In recent studies done in our lab by Adolphe et al. (2012, 2013, 2015), the brachial artery was used in dogs. This artery is more proximal than the median artery, but both are equally as easy to visualize. However, visualization of either forelimb artery in cats is difficult. After consulting with a veterinary radiologist from the University of Saskatchewan Veterinary Medical Centre, the abdominal aorta was determined to be more reliably imaged. Thus, in cats, the abdominal aorta is better for detection of endothelial dysfunction because it is a bigger artery in cats but does not permit the use of flow-mediated dilation techniques that the forelimb does. Thus, median artery in dogs and abdominal aorta pulse wave velocity and pulsatile distension in cats was used. Images of arteries are shown in Figure 2.3. With endothelial dysfunction (hypothesized to occur from elevated MG and ROS), arteries will become stiffer which will increase pulse wave velocity and decrease pulsatility (Marti et al. 2012).

2.8. Conclusions

Pulses offer a healthy alternative low GI carbohydrate source compared to high glycemic carbohydrates such as corn, rice and wheat. Few studies have looked at the digestibility of pulses and related this to glycemic responses in dogs and cats (Carciofi et al. 2008; de Oliveira et al. 2008). Pulses have been shown to have beneficial health effects in dogs, but whether this is true in cats is still undetermined. It is well known that there are species differences between cats and dogs in terms of dietary needs and metabolism.

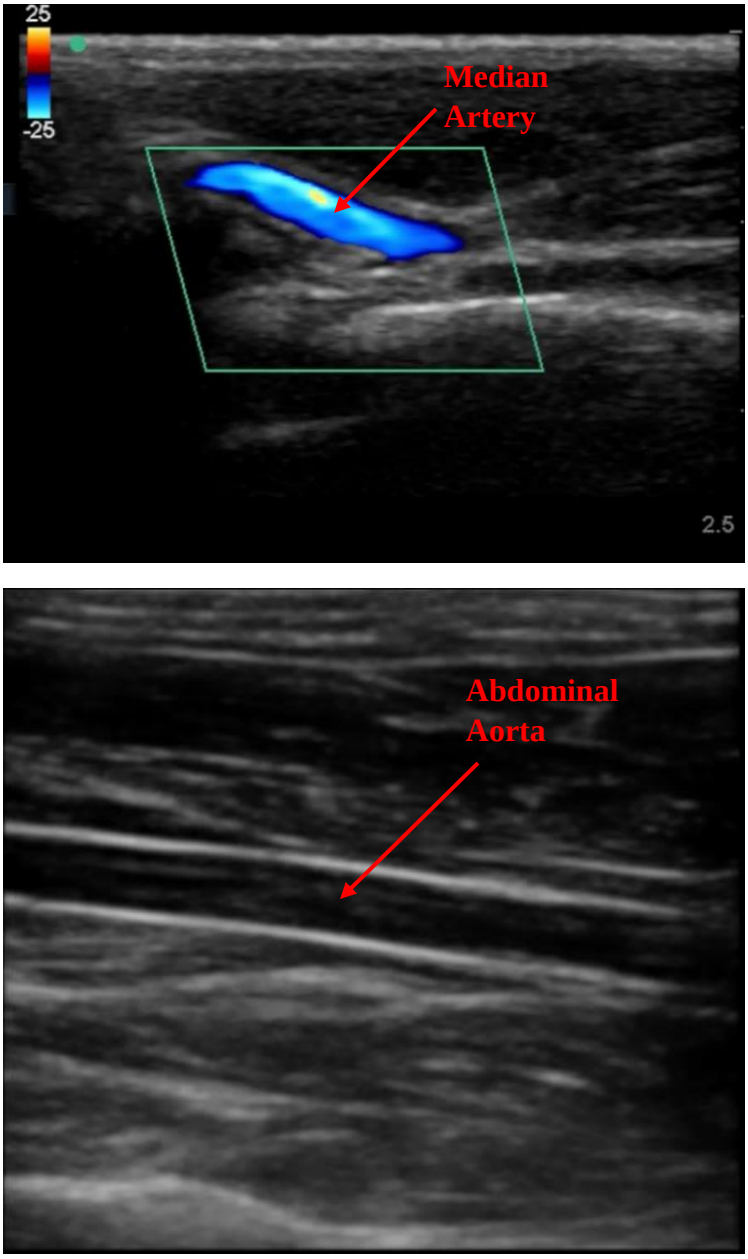


Figure 2.3 Ultrasound M-mode captured still images of dog median artery (top) and cat abdominal aorta (bottom) for analysis of arterial diameter.

What is less well understood and lacking in the literature is species differences in carbohydrate metabolism, in particular, to carbohydrate sources without any confounding factors such as fats and proteins. To determine this, a common method used in human nutrition studies is the area under the glucose and insulin response curves (AUC) and GI methodology. Whether this method is appropriate for use in species other than humans is still to be determined. Long-term consumption of high GI foods can lead to diseases such as diabetes and cardiovascular disease which are associated with reduced endothelial health and increased oxidative stress. Consumption of glucose has been shown to increase postprandial levels of MG in both humans and dogs, but this has not been determined in cats, or to whole formulated diet feeding tests. With the first study (Chapter 3) for this thesis I determined acute metabolic effects of pulse starches in dogs and cats and relate these to cardiovascular changes and methylglyoxal production. Then for the second study (Chapter 4) I evaluated long-term metabolic health effects of consumption of pulse-based diet versus a cornstarch diet and relate these to changes in cardiovascular parameters, methylglyoxal, and oxidative stress.

3. POSTPRANDIAL EFFECTS OF SINGLE FEEDINGS OF PURE STARCH AND WHOLE FORMULATED DIETS ON GLYCEMIC, INSULINEMIC, METHYLGLYOXAL, AND CARDIOVASCULAR RESPONSES IN OMNIVORES COMPARED TO CARNIVORES.

This study is the first part of the overall research work looking at effects of pulse starches as an alternative carbohydrate source in dogs and cats. This first study was to examine species differences and acute health effects of the starch ingredients alone and formulated into diets. This study will provide a better understanding of differences in carbohydrate metabolism between dogs and cats and he understand postprandial changes in methylglyoxal and cardiovascular parameters.

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Jennifer Briens did 100% of the animal work, data collection and data analysis for glucose, insulin, cardiovascular parameters, and oxidative stress as well as all writing of the manuscripts. Dr. Lynn Weber providing expertise and major editing of this thesis. Dr. Kaushik Desai contributed to methylglyoxal data collection by providing equipment, expertise and input for the HPLC work. Dr. Jennifer Adolphe contributed 100% to the diet formulations by providing us with balanced diets.

3.1. Introduction

Dogs and cats have become common family companions with more than 35% of households in Canada having four-legged family members (CVMA report. 2011). Nutrition is very important for companion animals. Because of this, pet owners are constantly looking for pet foods with long-term health benefits, although many of these claims may not be substantiated. An increasing number of pet owners are aware of the ingredients that go into pet food and are looking for transparency and traceability of the ingredients (Pet Food Industry. 2017). Since the 1980's trends in pet foods have closely resembled human health trends (CVMA report. 2011), influencing new product development such as grain free, using "natural" products or being environmentally sustainable (Agriculture and Agri-Food Canada. 2012).

With the grain free movement there is a desire to produce food with healthier alternative carbohydrate sources. Many pet owners see grains as fillers, question their need in diets, especially for cats, and perceive these as a low digestible carbohydrate sources in pet food (Laflamme et al. 2014). Pulses are an appealing option due to their low GI value in humans, higher levels of protein, classification as a non-grain carbohydrate source, non-genetically modified crops and sustainability due to their nitrogen fixing ability. Pulses have been reported to have GI values ranging from 40-60 in humans (Foster-Powell et al. 1995), which is lower than other common pet food ingredients such as corn which has a GI around 90-100 in humans. Glycemic index values for dogs have only been previously reported by our research group with the GI of unprocessed peas reported to be 29 ± 5, that of whole white rice 55 ± 6, and cornstarch 47 ± 10 (Adolphe et al. 2012). These are the only GI values to our knowledge that have been reported in dogs, and there are no values reported in cats. Not only is there a lack of GI index values reported for companion animals, we are unsure if GI has any meaningful physiological

benefits for species other than humans. Glycemic index values can also change due to processing effects, such as modification of the starch or diet formulation. Chung et al. (2008b) has shown that both hydroxypropylated and crosslinked cornstarch have increased levels of resistant starch, compared to unmodified cornstarch, which in turn decreases the estimated GI value. Adolphe et al. (2015) reported that the GI of both pea and rice increased when formulated and extruded into whole diets. It has been shown that when peas and beans are cooked the amount of rapidly digestible starches (RDS) increases due to gelatinization (Erayu et al. 2009) which could lead to more rapid digestion and higher glycemic response, but this has yet to be confirmed *in vivo*. There has been extensive research in both humans and animal models linking low GI foods with reduced incidence of post-prandial hyperglycemia as well as reduced incidence of chronic diseases such as obesity, insulin resistance, gestational diabetes and type 2 diabetes, and cardiovascular disease (Brand-Miller et al. 2009; Riccardi et al. 2008; Ludwig. 2002). Obesity is as much of a problem in companion animals as it is in humans and has recently been presented as a One Health problem due to our shared lifestyles and environmental factors (Chandler et al. 2017). Lund et al. (2005; 2006) reports that one in three dogs or cats that visit veterinary clinics are overweight or obese. Observations from a five-year study (2007-2011) showed that overweight and obese cases in dogs increased 37% and 90% in cats (Banfield. 2012). As with humans, 80% of reported diabetic cases in obese cats brought into veterinary clinics exhibit type 2 diabetes, where dogs are more likely to exhibit type 1 diabetes (Rand et al. 2004; Case et al. 2011).

Due to the fact that starch is one of the lowest cost ingredients in pet food, carbohydrates are most often the largest component. Species differences between cats and dogs has led to differences in the ability to metabolize carbohydrates (Batchelor et al. 2011). Cats, being

carnivores, have evolved to consume low carbohydrate diets, whereas dogs, being omnivores, can consume more diverse diets. Metabolic differences between these two species has been studied to some extent. Cats have been found to have lower intestinal levels of glucose transporter sodium-glucose linked transport type 1 (SGLT1) and has been suggested that cats have an inability to upregulate SGLT1 due to a lack of a sweet receptor subunit (Batchelor et al. 2011). Cats also have higher levels of rate limiting liver enzymes for gluconeogenesis than the dog (Washizu et al. 1999), suggesting that this is the main way they maintain their blood glucose levels when low carbohydrate carnivorous diets are consumed by wild cats. Along with this, cats also lack salivary amylase and have lower levels of pancreatic amylase than dogs, which can lead to decreased rates of carbohydrate breakdown (Meyer & Kienzle. 1991). Despite these differences in carbohydrate metabolizing enzymes, cats are thought to be more susceptible to hyperglycemia, which can lead to various disorders including diabetes mellitus. I determined metabolic responses to single feedings of pure starches, which has yet to be reported in the literature and as well the species differences in carbohydrate metabolism to determine if cats are in fact unable to utilize carbohydrates in their diets and if they become hyperglycemic.

Hyperglycemia can lead to the formation of toxic reactive glucose metabolites. Of particular concern is methylglyoxal (MG), a reactive dicarbonyl, which can lead to the formation of advanced glycation end products (AGEs). MG has been shown to play a role in the development of insulin resistance (Jia et al. 2006), abnormalities consistent with type 2 diabetes (Dhar et al. 2011), increased levels of oxidative stress and inflammation (Dhar et al. 2008) and arterial stiffening leading to increased blood pressure (Wang et al. 2008). Previous work from our laboratory was the first to show postprandial increases in MG levels in any species (dogs) following feedings of simple, but not complex carbohydrates in beagles (Adolphe et al. 2012).

The hypothesis for this study is that pulse starches and diets formulated with pulse starches would produce lower glycemic and insulinemic responses compared to commonly used grain-based pet food ingredients, such as cornstarch, in both dogs and cats. Cats are predicted to produce higher glycemic and insulinemic responses to the carbohydrates compared to dogs, and in turn this will result in higher GI values. Higher glycemic responses in the cats compared to dogs were predicted to cause higher postprandial MG levels, leading to oxidative stress that would decrease vasodilation, impair endothelial function, increase blood pressure and increase pulse wave velocity. In order to test this hypothesis, laboratory-fasted beagle dogs and mixed breed domestic cats were fed single, standard-sized meals of test starches alone or formulated in whole diets to evaluate glycemic, insulinemic, cardiovascular, and MG responses. The starch sources chosen were either from cereals, tubers or pulses. These starch sources were chosen based on human studies, to represent both high and low GI starches. Of note, the high GI starches determined in human studies are often the carbohydrate sources used in pet foods. I also tested a modified versus unmodified cornstarch to determine if modifications would produce a lower glycemic response and GI similar to that of pulse starches. Modifications can be done to starches to change certain properties, such as thickening and gelling and have been shown to have increased levels of resistant starch compared to the same unmodified starch (Chung et al. 2008b). Corn was chosen as a control starch source to compare to diets with pulse starches, due to the fact that cornstarch is a high GI carbohydrate in humans and is a common inexpensive ingredient for pet food. Pulse starches were chosen to represent a low GI starch source.

3.2. Materials and Methods

3.2.1. Animals

Mixed breed domestic cats (n=8, three males and five females; neutered/spayed; 2-3 years old) and beagle dogs (n=8, four males and four females; neutered/spayed; 2-3 years old) were obtained from a certified scientific breeder. The cats were able to roam freely during the day and housed individually in kennels during feeding and at night in the Animal Care Unit (ACU) at the Western College of Veterinary Medicine (WVCM). The dogs were group-housed in a separate dog room during the day with access to outdoor runs and housed in individual kennels during feeding and at night. The dogs were either walked daily or allowed access to a fenced outdoor dog park located behind the WCVM. During the trials, except for during feeding experiments, the animals were fed a standard commercial dry pet food (Hill's Science Diet, Hill's Pet Nutrition, Inc. Topeka, USA). The dogs were fed two meals a day, once in the morning and once in the early evening, while the cats were fed one meal a day, in the early evening. The amount the animals were fed was based on that required to maintain ideal body condition scores in each animal using the National Research Council guidelines (National Research Council. 2006). Average body condition scores were between 4-5 based on a 9-point scale (Laflamme. 1997a, 1997b). Blood samples were collected from each animal prior to the study and sent to Prairie Diagnostic Centre, located in the WCVM, for basic small animal blood chemistry panel, and complete blood count (CBC) analysis, to get baseline measurements and ensure all animals were in good health during trials. Fecal quality was assessed qualitatively and collected at random for glucose analysis. All animal handling and procedures were conducted according to a protocol approved by the University of Saskatchewan Animal Research Ethics Board under the guidance of the Canadian Council on Animal Care for humane animal use.

3.2.2. Starch and Glucose Control Postprandial Testing

In vitro available carbohydrate (free sugars plus starch) was determined for all starch sources and diets using a commercially available kit (Megazyme International, Ireland) which uses α-amylase and amyloglucosidase to produce hydrogen peroxide (H_2O_2). The H_2O_2, along with peroxidase, forms a quinoneimine dye which is then quantitatively measured using a spectrophotometer to assess total available carbohydrate content. These results were then used to calculate how much of the starches and complete diets should be fed to provide a standardized amount of available carbohydrate to each animal. The animals were fed 1g of available carbohydrate per kg body weight respective to each individual animal. The oral glucose solution was fed at 1g per kg body weight per animal and tested in duplicate. The glucose and starches were combined with water to make a solution which was then placed into a syringe and to feed each animal. The diets were fed either in kibble form or ground and mixed with water to form a slurry if animals would not willingly eat the diets. The dogs consumed all starches and diets within 5 ± 2 minutes, and the cats consumed all starches and diets in 7 ± 2 minutes. Post-prandial responses to glucose (positive control) or single feedings of starches: unmodified store cornstarch (Fleischmann's, AB Mauri Limited, Ontario, Canada), modified (hydroxypropyl substituted cross linked) cornstarch (Tate & Lyle, HQ London, UK), yellow pea flour (dry processed, AGT Foods, Regina, CA), fava bean flour (dry processed, AGT Foods, Regina, CA), yellow lentil flour (dry processed, AGT Foods, Regina, CA), rice flour (Deep Foods, Inc. NJ, USA), tapioca starch (Ingredion, HQ Westcheester, IL, USA), white wheat flour (Robin Hood, Smucker Foods of Canada Corp., ON, Canada), potato starch (Meelunie B.V., Amsterdam, Netherlands) were randomly assigned and tested sequentially in all animals (n=8 dogs; n=8 cats). Animals were tested only once per week to prevent significant blood loss and associated anemia. Whole diet formulations are in Table 3.1.

Table 3.1 Diet formulations for nutritionally complete diets with 30% inclusion of modified corn, pea, faba bean or lentil starch used in testing for both cats and dogs.

Ingredient	Corn Diet (%)	Pea Diet (%)	Faba bean Diet (%)	Lentil Diet (%)
Cornstarch	30.00	-	-	-
Pea Starch	-	30.00	-	-
Faba bean Starch	-	-	30.00	-
Lentil Starch	-	-	-	30.00
Chicken meal	35.78	25.46	23.76	24.95
Soy Protein concentrate	9.31	15.00	15.00	15.00
Chicken Fat with Dadex[1]	7.75	9.76	10.44	9.84
Pea Fibre	4.96	6.88	6.89	6.90
Fish meal, mixed	5.00	5.00	5.00	5.00
AFB LC647[2]	2.00	2.00	2.00	2.00
Fish Oil	2.00	2.00	2.00	2.00
Potassium Chloride	0.90	0.88	1.00	1.00
Celite	1.00	1.00	1.00	1.00
Sodium Chloride	0.30	0.50	0.50	0.50
AFB F24047 Dry[2]	0.50	0.50	0.50	0.50
Calcium carbonate	0.00	0.41	0.55	0.43
Choline chloride	0.10	0.20	0.43	0.39
Taurine	0.10	0.10	0.10	0.10
Methionine D/L	0.10	0.10	0.10	0.10
Mineral Premix (Dog/Cat)[3]	0.10	0.10	0.10	0.10
Vitamin Premix (Cat)[3]	0.10	0.10	0.10	0.10
Dicalcium Phosphate	-	0.01	0.53	0.09

[1]Dadex = an antioxidant solution for animal fats to help extend shelf life of ingredient and pet food

[2]Dry palatant: Dry cat palatability enhancer BioFlavor F24047, Wet palatant: Liquid Cat palatability enhancer, Optimizor LC 647. Added to improve palatability of diets.

[3]Wheat, magnesium oxide, zinc, methionine, vitamin C, Alltech Bio-Mos, vitamin E, zinc sulphate, ferrous sulphate, iron proteinate, vitamin D3, Alltech deodorase, mineral oil, copper proteinate, copper sulphate, niacin, selenium enriched yeast, calcium iodate, vitamin A, manganese proteinate, calcium pantothenate, biotin, vitamin B12, riboflavin, manganese oxide, thiamine, sodium selenite, pyridoxine, folic acid.

Diets were formulated using Concept 5 software (Creative Formulation Concepts, LLC, MD, USA) according to AAFCO nutrient profiles (The Association of American Feed Control Officials. 2014) and performed by Dr. Jennifer Adolphe (Senior Nutritionist, Petcurean, Chilliwack, BC, Canada). The diets were the same for both dog and cat and formulated to meet nutritional requirements for maintenace of adult cats (The Association of American Feed Control Officials. 2014). The diets were extruded at the Saskatchewan Food Industry Development Centre located at the University of Saskatchewan (Saskatoon, SK, Canada), using a Clextral Evolum 32 twin screw extruder (Firminy, France) with a 24:1 length diameter ratio and a 2.88 mm die. Proximate analysis of the diets are included in Chapter 4 (Table 4.4).

3.2.3. Blood Collection

Animals fasted overnight were aseptically catheterized using an intravenous catheter inserted into the cephalic vein each day of testing. Blood samples were collected (2 ml for dogs; 1 ml for cats) and placed into tubes containing K2 EDTA and centrifuged for five minutes at 5600 rpm (Statspin Express 3, Beckman Coultier Inc., USA) to separate plasma for analysis. Emla cream (2.5% Lidocaine, AstraZeneca, Mississauga, CA) was needed for the cats only as a topical anaesthetic to minimize stress and movement during catheterization. No other anaesthetics or sedatives were used in this study. Plasma was aliquoted and then stored at -80°C until used in analyses. In preliminary experiments, the time to peak and time to return to baseline of postprandial glycemic responses was determined to take longer in cats compared to dogs. Therefore, blood was collected for the dogs at 0 (pre-feeding) and 15, 30, 45, 60, 90, 120, 150, 180 minutes postprandial (Adolphe et al. 2012); and for the cats at 0 (pre-feeding) and 15, 30, 60, 120, 180, 240, 300 minutes postprandial. Following collection, catheters were flushed with a

1 ml 3% sodium citrate (Omnipure, Darmstadt, Germany) in 0.9% NaCl (Sigma Aldrich, St.Louis, USA) sterile solution to ensure patency of catheter and for fluid replacement. After testing was finished on a given day, catheters were removed, animals fed their normal meal and returned to normal husbandry.

3.2.3.1. Glucose analysis

Plasma and fecal glucose analysis was performed using a glucose oxidase assay method using reagents from Sigma Aldrich (St.Louis, USA) and analyzed on a Spectra Max 190 Microplate reader (Molecular Devices, LLC. Sunnyvale, USA). Plasma glucose was determined at all time points for all treatments. To measure the GI of each of the starches and the whole formulated diets, incremental AUC was calculated using the trapezoid rule, and divided by the average glucose AUC for each respective animal (Wolever et al. 1991). Peak glucose levels and time to peak were also calculated for each glucose control, starch or diet tested. Fecal glucose was determined for randomly selected fecal samples.

3.2.3.2. Insulin analysis

Plasma insulin analysis was performed using enzyme linked immunosorbent assays purchased from Mercodia Inc. (Uppsala, Sweden). A canine specific ELISA was used for the canine plasma samples, while a human insulin specific ELISA, validated for use in cats by the manufacturer, was used for the feline samples. Results were analyzed on a Spectra Max 190 Microplate reader. Plasma insulin was determined at all times points for all treatments and incremental AUC was calculated for each treatment using the trapezoid rule (Wolever et al. 1991). Peak insulin levels and time to peak were also calculated for glucose plus every starch or diet tested.

3.2.3.3. Methylglyoxal

Plasma methylglyoxal was analyzed using high performance liquid chromatography (HPLC) methods previously described (Wang et al. 2005) and validated in dogs in our research group (Adolphe et al. 2012). Two samples were analyzed per animal per treatment, one pre-feeding (time 0) and one at 60 minutes postprandial. Results were expressed as the change in MG plasma levels from pre-feeding to 60 minutes postprandial.

3.2.4. Cardiovascular Parameters

Blood pressure and heart rate were determined by high definition oscillometry using a Vet Memodiagnostic HDO monitor (S + B medVET, Germany) and analyzed using MDS Win Analyse software version 2.1.2.1. The cuff was placed at the base of the tail, and measurements were taken at time 0 (pre-feeding) and 60 minutes postprandial. An average of three measurements were used for analysis of systolic and diastolic pressures and heart rate at each time, as previously validated in our lab in dogs (Adolphe et al. 2012).

3.2.5. Statistical Analysis

Data was analyzed using IBM SPSS version 20.0 (International Business Machine Corp., USA). Two-way repeated measures ANOVA was used to determine differences between species as well as among treatments for peak, time to peak, AUC, and GI. Pairwise differences were detected in post hoc analyses using Fisher's least significant difference (LSD). A $p<0.05$ will be considered significant. When data failed ANOVA assumptions, such as time to peak in the cats following the single feedings of the starches which showed heteroscadicity in variance, I used a Friedman's test for repeated measures using a Tukey multiple comparison test to determine any pairwise differences. For species comparisons within a treatment, an independent samples t-test

was used to evaluate significant differences. Cardiovascular parameters were analyzed using paired samples t-test due to samples being taken from the same animal within the same treatment across different time points. Species differences were analyzed using independent samples t-test to evaluate differences within a treatment.

3.3. Results

3.3.1. *In Vitro* Available Carbohydrate

The available carbohydrate values for the starch sources and whole diets are found in Table 3.2. The available carbohydrate content was highest for the potato starch, tapioca starch, unmodified cornstarch, and rice flour, while the pulse starches and the modified cornstarch produced the lowest values. This shows that the tuber starches have the highest available carbohydrate content (100% available carbohydrate), followed by the cereal starches (82-100% available carbohydrate), and lastly the pulse starches (71-76% available starch). The modified cornstarch was lowest (67% available starch) and was used as the carbohydrate source for the control diet formulation since it had an available carbohydrate content similar to the pulse starches. A similar trend was seen with the whole diets, in that the modified cornstarch diet had the lowest amount of available carbohydrate (24%) compared to the pulse-based diets (26-28%). Out of the three pulse-based diets, the lentil diet had the highest amount of available carbohydrate, with pea and faba bean showing very similar results. These results were then used to standardize the single feeding tests so each starch meal consumed contained the same amount of available carbohydrate.

Table 3.2 In vitro available carbohydrate of the starch sources and formulated diets with 30% inclusion of modified corn, pea, faba bean or lentil starch.

Carbohydrate source (CHO)	Total available CHO (g/100g)	Amount for 1g available CHO
Potato	108.50	1.00g
Tapioca	105.44	1.00g
Unmodified Cornstarch	101.14	1.00g
Rice	97.38	1.03g
Wheat	81.70	1.22g
Lentil	75.89	1.32g
Pea	75.28	1.33g
Faba bean	71.23	1.41g
Modified Cornstarch	65.95	1.52g
Lentil diet	28.68	3.49g
Faba bean diet	26.55	3.77g
Pea diet	26.45	3.78g
Modified cornstarch diet	24.01	4.17g

Starches and diets are shown in descending order from highest to lowest of total available CHO

3.3.2. *In Vivo* Glucose and Insulin Responses

Plasma samples collected over three hours (dogs) or five hours (cats) were analyzed for glucose and insulin responses. Time course results for both species are shown in Figure 3.1, while peak, time to peak, AUC and GI results are shown in Table 3.3 (glucose) and Table 3.4 (insulin). The results are shown in decreasing GI order for dogs, with a rank order from highest to lowest: tapioca starch, white wheat flour, rice flour, modified cornstarch, pea starch, lentil starch, faba bean starch, and finally potato starch (Table 3.3). In order to more directly compare between species, results for starch sources in cats were listed in the same order as the dogs in Tables 3.3 and 3.4, even though the GI responses and order from highest to lowest differed. For cats, the GI rank order from highest to lowest were: rice flour, white wheat flour, potato starch, modified cornstarch, tapioca starch, lentil starch, faba bean starch, and pea starch. In dogs there were no significant differences in the GI values among any of the starch sources. However, the glucose control produced a significantly higher peak in plasma glucose (mmol/L), compared to all other starches, followed by rice flour and unmodified corn.

The pulse starches, in dogs, produced peak plasma glucose values that were significantly lower than rice flour, but did not differ significantly compared to potato starch, tapioca starch and white wheat flour. Time to peak (min) for blood glucose was significantly longer for the pulse starches (pea, lentil and faba bean) and potato starch, compared to the glucose control and rice flour. The dog AUC of the postprandial plasma glucose response for the pulse starches were significantly lower compared to the glucose control, but there were no significant differences seen among the starch sources. The decreasing rank order for the starch source postprandial glucose AUC values in dogs are tapioca, white wheat flour, rice flour, unmodified cornstarch, pea, modified cornstarch, lentil, faba bean and potato.

Table 3.3 Postprandial glycemic responses in fasted dogs and cats following a single feeding of a glucose control (15% w/v solution; 1 g/kg) compared to single feedings of pure starches (1g available carbohydrate/kg bodyweight) from different sources.

	Glucose	Tapioca	Wheat	Rice	UnMod. Corn	Pea	Mod. Corn	Lentil	Faba bean	Potato
DOGS										
Peak (mmol/L)	6.82 ± 0.3 ^{a}	4.8 ± 0.2 ^{c,d,e}	4.74 ± 0.2^{c,d,e}	5.50 ± 0.1^{b}	5.24 ± 0.2 ^{b,c}	4.74 ± 0.1^{c,d}	4.41±0.1 ^{d,e}	4.38 ± 0.1 ^{d,e}	4.31 ± 0.1 ^{e}	4.49 ± 0.1^{c,d,e}
Time to Peak (min)	35 ± 2 ^{a}	51 ± 6 ^{b,c}	47 ± 8 ^{a,b,c}	36 ± 4 ^{a,b}	49 ± 7 ^{a,b,c}	53 ± 4 ^{c}	41 ± 4 ^{a,b,d}	54 ± 4 ^{c}	60 ± 8 ^{c,d}	49± 5 ^{c,d}
AUC (mmol/L min)	111 ± 16 ^{a}	100 ± 29^{a,b}	57 ± 12^{b}	57 ± 15^{b}	55 ± 22^{a,b}	52 ± 16 ^{b}	49 ± 9 ^{b}	49 ± 10 ^{b}	45 ± 16 ^{b}	42 ± 24 ^{b}
GI		93 ± 32	56 ± 16	55 ± 16	55 ± 26	49 ± 15	48 ± 11	47 ± 10	46 ± 17	34 ± 19
CATS										
Peak (mmol/L)	6.90 ± 0.5^{a}	3.93 ± 0.1^{c,d}	4.20 ± 0.1^{c}	4.84 ± 0.2^{b}	5.17 ± 0.2^{b}	3.90 ± 0.1^{d}	3.91 ± 0.1 ^{d}	3.94 ± 0.1^{d}	3.79 ± 0.1^{d,e}	3.56 ± 0.1^{e}
Time to Peak (min)	57 ± 2^{a,b}	60 ^{a,b}	90 ± 11^{a}	56 ± 4^{a,b}	39 ± 8 ^{b}	45 ± 6 ^{a,b}	56 ± 4 ^{a,b}	79 ± 13^{a,b}	41 ± 6 ^{b}	71 ± 11^{a,b}
AUC (mmol/L min)	224 ± 28^{a}	58 ± 19^{c,d,e}	92 ± 17^{b,c}	100 ± 13^{b}	57 ± 6^{c,d,f}	13 ± 4 ^{e,g}	42 ± 12^{c,f}	29 ± 8^{e,f,g}	12 ± 4 ^{g}	75 ± 17^{b,d}
GI		25 ± 6 ^{b,c,d}	41 ± 5 ^{a,b}	47 ± 6 ^{a}	29 ± 5 ^{b,c,d}	6 ± 2 ^{e,f}	21 ± 6 ^{b,d,e}	15 ± 4 ^{d,e,f}	6 ± 2 ^{f}	35 ± 7 ^{a,c}

Results are shown in descending order of glycemic index (GI) values for dogs. Values are mean ± SEM; n = 8 (dogs), n = 8 (cats). Values in a row with superscripts without a common letter differ; $p < 0.05$, one-way repeated measures ANOVA with Least square difference post hoc test, except for time to peak for the cats where a Friedman's test for repeated measures was used due to violations of parametric assumptions. AUC = area under the curve for the postprandial plasma glucose response. UnMod Corn = unmodified cornstarch, Mod. Corn = Modified cornstarch.

Table 3.4 Postprandial insulinemic responses in fasted dogs and cats following a single feeding of a glucose control (15% w/v solution; 1 g/kg) compared to single feedings of pure starches (1g available carbohydrate/kg bodyweight) from different sources.

	Glucose	Wheat	Rice	Lentil	Tapioca	UnM Corn	Pea	Faba bean	Mod Corn	Potato
DOGS										
Peak (pmol/L)	188±28^{a}	76±14^{c,d}	99±12^{b}	61±9^{c,d,e}	40±5^{e,f}	42±5^{c,e,f}	61±11^{c,d,e,f}	60±5^{d}	39±3^{f}	43±13^{c,d,e,f}
Time to Peak (min)	30±3^{a,b}	30±4^{b}	28±4^{a,b}	34±6^{a,b}	38±7^{a,b}	47±10^{a,b}	26±3^{a,b}	38±6^{a,b}	26±5^{a,b}	45±9^{a}
AUC (pmol/L min)	5703±940^{a}	4527±1268^{a,b,c}	3667±507^{b}	2513±774^{b,c,d}	2329±494^{b,c,e}	2249±392^{c,e}	2122±512^{c,e}	2000±337^{c,e}	1747±725^{d,e}	809±210^{d}
CATS										
Peak (pmol/L)	111 ± 13^{a}	37 ± 5^{d}	54 ± 5^{b}	37 ± 5^{c,d}	20 ± 3^{e}	47 ± 4^{b,c,d}	48 ± 5^{b,c,d}	53 ± 8^{b,c}	23 ± 4^{e}	16 ± 2^{e}
Time to Peak (min)	50 ± 11^{a,b}	39 ± 6^{a,b}	30 ± 5^{a,b,,c}	15^{d}	60 ± 15^{a}	26 ±3^{b}	19 ± 2^{c,d}	19 ± 2^{d}	30 ± 13^{a,b,,c,d}	39 ± 8^{a,b}
AUC (pmol/L min)	8525 ± 1073^{a}	2505 ± 600^{b,c}	3330 ± 442^{b}	1818 ± 491^{c,d}	751 ± 133^{d}	2086 ± 695^{b,c,d}	2615 ± 382^{b,c}	1519 ± 456^{c,d}	1009 ± 264^{d}	924 ± 284^{d}

Results are shown in descending order of insulinemic postprandial area under the curve (AUC) value for dogs; cat insulinemic AUC values are aligned with the dog. Values are mean ± SEM; n = 8 (dogs), n = 8 (cats). Values in a row with superscripts without a common letter differ; $P < 0.05$, one-way repeated measures ANOVA with least square difference (LSD) post hoc test. UnM Corn = unmodified cornstarch. Mod Corn = modified cornstarch.

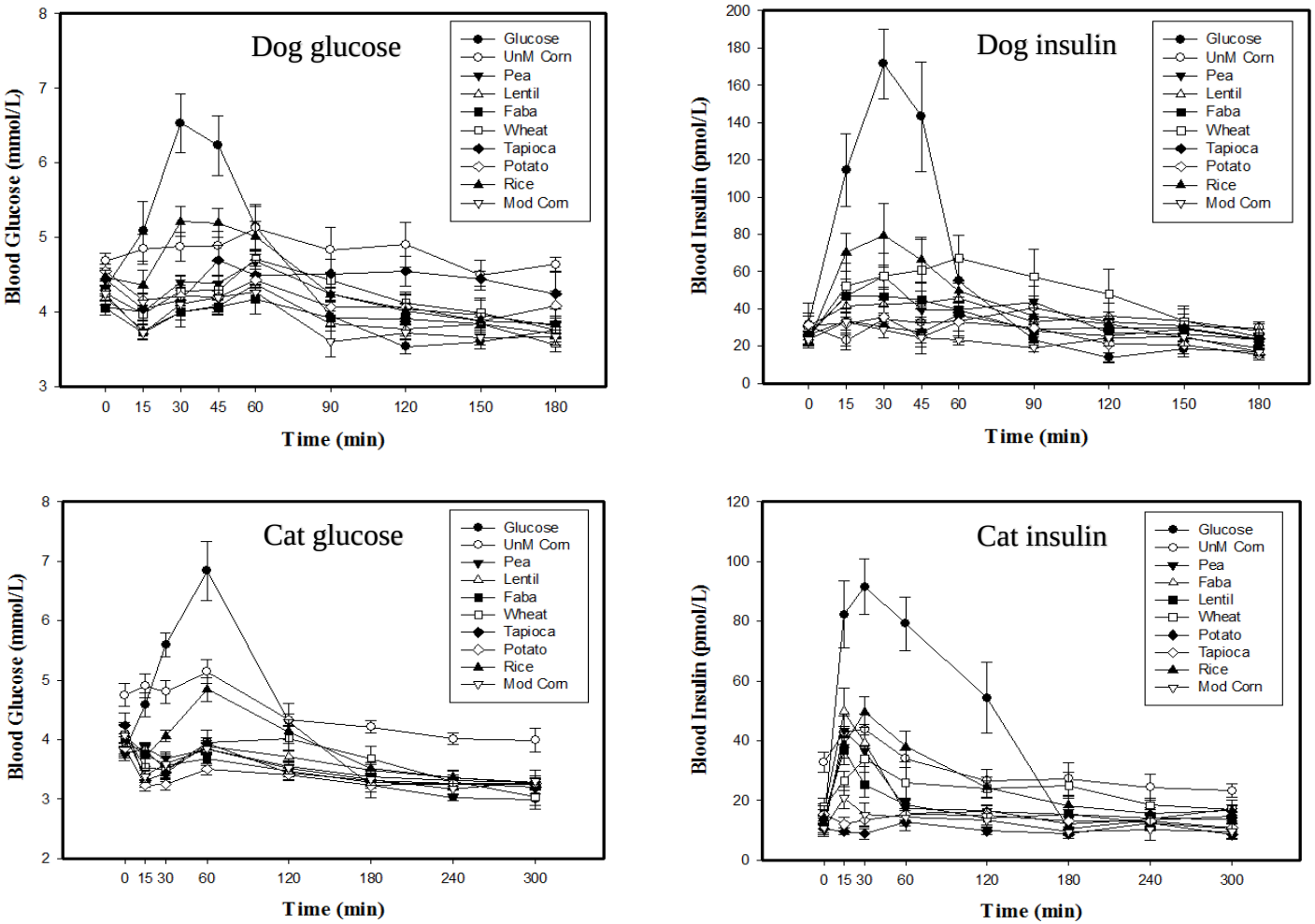

Figure 3.1 Blood plasma glucose and insulin time course curves following single feedings of starches and a glucose control. Fasted dogs (n=8) and cats (n=8) were fed 1 g available carbohydrate per kg body weight (BW) of each starch or a glucose control (15% w/v solution). Values are mean ± SEM

The results from the cats showed that rice flour produced the highest GI, which is significantly higher than all starches except white wheat flour and potato starch. The pulse starches produced the lowest GI results of which pea and faba bean were significantly lower than all other starches except lentil. The peak in plasma glucose (mmol/L) was significantly higher in the glucose control compared to all the starches. When comparing the starch sources unmodified cornstarch and rice flour have a significantly higher peak and potato produced the lowest peak, only not significantly lower than faba bean. White wheat flour produced the longest time to peak, while the shortest time to peak was unmodified corn. Only white wheat flour had a significantly longer time to peak plasma glucose than faba bean and unmodified corn, while there are no significant differences seen among the rest of the starches. The pulse starches varied with faba bean and pea producing a shorter time to peak in plasma glucose than lentil. The postprandial blood glucose AUC for the glucose control was significantly higher than all of the starch sources, but in terms of the starch sources, rice flour produced the highest AUC and pea and lentil produced the lowest AUC. Postprandial blood glucose AUC for rice flour, white wheat flour and potato starch were all significantly higher than the pulse starches AUC values.

Comparing acute plasma glucose responses between species for the starch testing, peak plasma glucose responses were generally surprisingly similar between the two species (Table 3.3). Despite the similarity, peak glucose was significantly lower in cats compared to dogs after feeding all starches except for the glucose control and unmodified cornstarch. Of note, fasting cat plasma glucose was 3.60 ± 0.09 (Figure 3.1), and postprandial responses to all of the pulse starches remained at or below this fasting level for all times tested, while dogs did exhibit a clear peak glucose to these pulse starches (dog fasting plasma glucose was 4.32 ± 0.06), albeit low

compared to other starches (Figure 3.1). Time to peak plasma glucose was significantly longer in cats compared to dogs when fed the glucose control, white wheat flour, rice flour, and modified cornstarch (Figure 3.1). There were no other species differences in times to peak glucose. Postprandial plasma glucose AUC results showed cats have significantly higher AUC for the glucose control and rice flour, while the pea starch produced a significantly lower postprandial plasma glucose AUC in cats compared to dogs (Table 3.3). No other starches showed significant differences between species for glucose AUC. Glycemic index values for cats are significantly lower for pea ($p=0.025$), lentil ($p=0.014$) and modified cornstarch ($p=0.042$), compared to the dog GI values for these same starches. All other starches showed no species differences for GI, although faba bean starch GI between species was close to achieving significance at $p = 0.051$ (Table 3.3).

Dog postprandial plasma insulin peak values (pmol/L; Table 3.4), were significantly higher for glucose than all the starches. Rice flour produced the second highest postprandial insulin peak which was significantly lower than glucose but significantly higher than all the other starches. In dogs, tapioca starch produced the lowest peak which was significantly lower than glucose, rice flour, white wheat flour and faba bean starch. The AUC for postprandial insulin response in dogs followed the same trend as the plasma glucose results with glucose, white wheat flour and rice flour producing significantly higher values than modified cornstarch and potato starch. Potato starch in dogs produced the lowest postprandial insulin AUC, a trend similar to that observed for postprandial glucose responses to this starch in dogs, but this postprandial insulin AUC value was not significantly different from the lentil starch.

Cat postprandial plasma insulin peak value for the glucose control was significantly higher than all of the other starches (Table 3.4). Postprandial insulin responses in cats to potato

and tapioca starches (tuber starches) produced the lowest peaks which were significantly lower than all other starches. Of all the pulse starches, lentil starch produced the lowest postprandial insulin peak in cats and was the only pulse starch significantly lower than rice flour. The fastest time to postprandial insulin peak (min) was seen following pulse starches, namely lentil, pea and faba bean in cats, which were all significantly shorter than glucose or tapioca starch. In fact, time to peak postprandial insulin response was longest for tapioca starch in cats. The postprandial insulin AUC response for the glucose control was significantly higher than all other starches in cats. The next highest postprandial insulin AUC response in cats was for rice flour, which was significantly higher than for lentil, faba bean, potato and tapioca starches.

Comparing between species, dogs have significantly higher peak postprandial insulin levels compared to cats for the glucose control and all starches except for unmodified cornstarch, pea starch, faba bean starch and potato starch (Figure 3.1). Fasting plasma insulin levels for cats were 15.34 ± 1.98 pmol/L, where as dogs had significantly higher levels at 27.50 ± 1.10 pmol/L, showing that cat values start and remain lower than dogs. Postprandial time to peak insulin results only showed significant species differences with the three pulse starches (pea $p=0.049$; lentil $p=0.011$; faba bean $p=0.022$), with cat time to peak insulin being significantly shorter than for dogs. Postprandial insulin AUC values were only significantly different between species for the glucose control ($p=0.035$), in which the cats were significantly higher than the dog, and tapioca starch ($p=0.015$), in which the cats were significantly lower than the dog (Figure 3.1).

The amount of whole diet formulated with 30% modified cornstarch or pulse starches (pea, lentil and faba bean), fed to dogs and cats for postprandial glycemic and insulinemic responses were normalized to feed 1g available carbohydrate per kg body weight in each meal. Time course of the plasma glucose and insulin results after feeding whole diets are shown in

Figure 3.2, and summary data is shown in Table 3.5 for glucose responses and Table 3.6 for insulin responses. In dogs, the postprandial plasma peak glucose response to feeding the glucose control versus diets are shown in decreasing rank order from highest response to lowest: glucose control > modified cornstarch diet > lentil diet > faba bean diet > pea diet. In dogs, the postprandial plasma glucose response to feeding the glucose control also produced the shortest time to peak, but was not significantly different from the time to postprandial glucose peak after feeding the lentil diet. In dogs, significantly higher postprandial glucose AUC response was observed after feeding the glucose control compared to all whole diets (modified corn, pea, lentil or faba bean starches). In dogs, the rank order for GI responses to the whole formulated diets are as follows: modified cornstarch diet > pea diet > faba bean diet > lentil diet, although none of the diets GI values were significantly different from each other.

For cats, postprandial plasma glucose after feeding the glucose control produced a significantly higher peak and AUC than all test diets (modified corn, pea, lentil or faba bean; Table 3.5). Postprandial peak plasma glucose and AUC response for all diets in cats were not significantly different from each other. For the time to peak postprandial glucose results in cats, there were no significant differences among the glucose control and all four test diets. Moreover, there were no significant differences among the GI values obtained in cats for whole diets tested but showed the following rank in decreasing order: modified cornstarch > pea > lentil > faba bean.

Table 3.5 Postprandial blood glucose responses from dogs and cats following single feedings of a glucose control (15% w/v solution; 1g/kg) and whole diets formulated with 30% inclusion of the corresponding starch (meals fed to give 1g available carbohydrate/ kg bodyweight).

	Glucose	Modified Cornstarch Diet	Pea Starch Diet	Faba Bean Starch Diet	Lentil Starch Diet
DOGS					
Peak (mmol/L)	6.8 ± 0.3 [a]	5.3 ± 0.1 [b]	4.7 ± 0.1 [c]	4.8 ± 0.2 [c]	5.0 ± 0.1 [b,c]
Time to Peak (min)	35 ± 2 [a]	53 ± 4 [b]	51 ± 5 [b]	64 ± 6 [b]	58 ± 10 [a,b]
AUC (mmol/L min)	111 ± 16 [a]	61 ± 11 [b]	49 ± 13 [b]	47 ± 9 [b]	36 ± 12 [b]
GI		65 ± 15	55 ± 20	48 ± 11	37 ± 11
CATS					
Peak (mmol/L)	7.0 ± 0.4 [a]	4.1 ± 0.2 [b]	3.9 ± 0.1 [b]	4.1 ± 0.2 [b]	3.9 ± 0.1 [b]
Time to Peak (min)	58 ± 2	43 ± 7	56 ± 4	49 ± 6	69 ± 12
AUC (mmol/L min)	217 ± 23 [a]	67 ± 17 [b]	57 ± 13 [b]	67 ± 23 [b]	58 ± 7 [b]
GI		33 ± 7	30 ± 9	29 ± 7	30 ± 4

Results are shown in descending order of glycemic index (GI) values for dogs. Values are mean ± SEM; n = 8 (dogs), n = 8 (cats). Values in a row with superscripts without a common letter differ; $p < 0.05$, one-way repeated measures ANOVA with least square difference (LSD) post hoc test. AUC = area under the curve for the postprandial plasma glucose response.

Table 3.6 Postprandial blood insulin responses from dogs and cats following single feedings of a glucose control (15% w/v solution; 1g/kg) and whole diets formulated with 30% inclusion of the corresponding starch (meals fed to give 1g available carbohydrate/ kg bodyweight).

	Glucose	Modified Cornstarch Diet	Pea Starch Diet	Faba Bean Starch Diet	Lentil Starch Diet
DOGS					
Peak (mmol/L)	187 ± 28 [a]	126 ± 14 [b]	92 ± 10 [b,c]	96 ± 11 [b,c]	91 ± 10 [c]
Time to Peak (min)	30 ± 3 [a]	51 ± 8 [a,b]	56 ± 10 [b]	68 ± 7 [b]	54 ± 7 [b]
AUC (mmol/L min)	5703 ± 911	8172 ± 1387	5946 ± 775	5840 ± 520	5093 ± 977
CATS					
Peak (mmol/L)	85 ± 12 [a]	59 ± 10 [a,b]	64 ± 12 [a,b]	51 ± 11 [b]	49 ± 10 [b]
Time to Peak (min)	61 ± 11	47 ± 12	39 ± 6	47 ± 12	47 ± 12
AUC (mmol/L min)	6478 ± 644	6074 ± 951	5847 ± 1276	4777 ± 1323	4068 ± 1033

Results are shown in descending order of postprandial insulin area under the curve (AUC) value of the diets for dogs; cat AUC values are aligned with the dog. Values are mean ± SEM; n = 8 (dogs), n = 8 (cats). Values in a row with superscripts without a common letter differ; $p < 0.05$, one-way repeated measures ANOVA with least square difference (LSD) post hoc test.

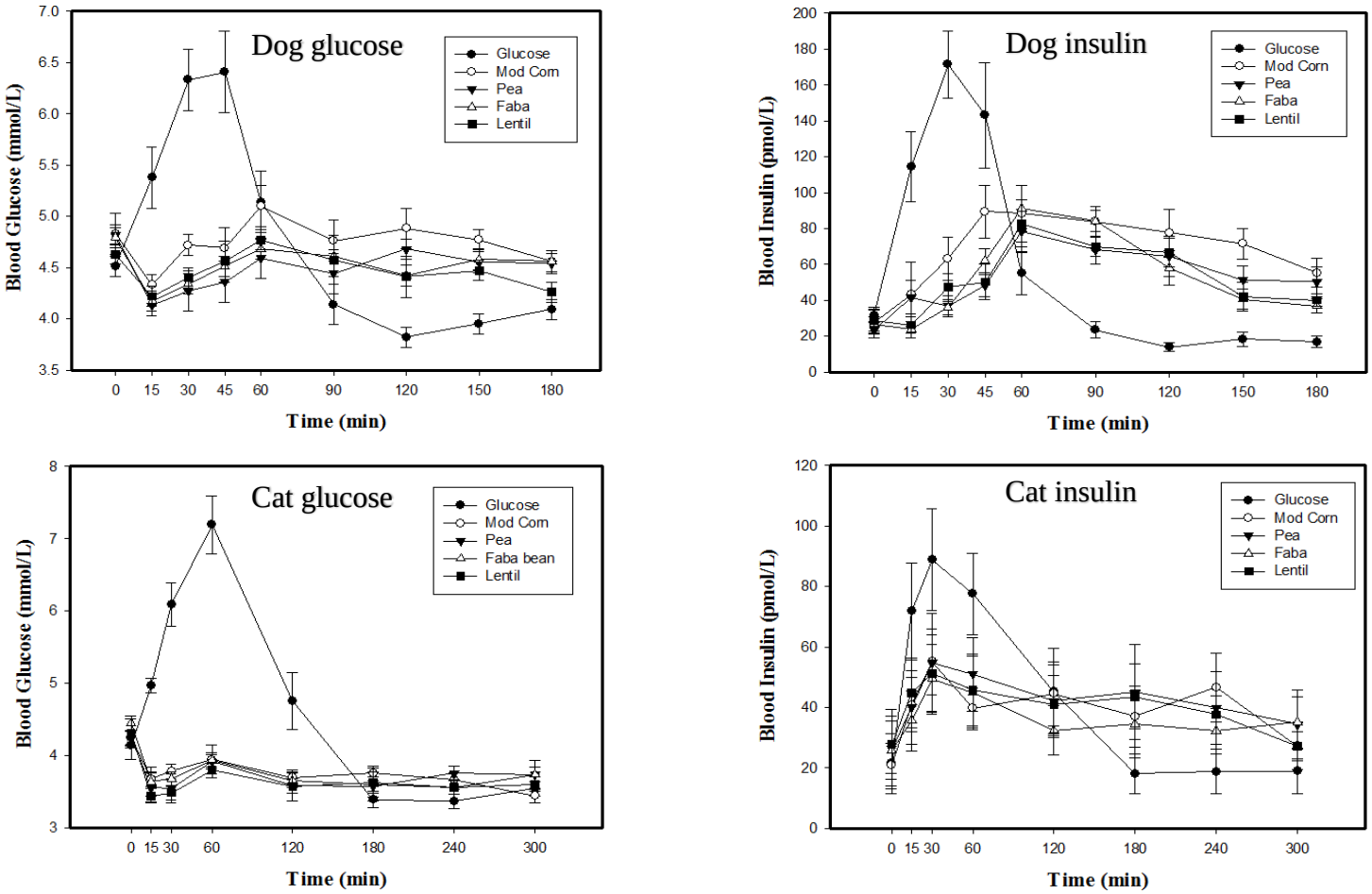

Figure 3.2 Blood plasma glucose and insulin time course curves following single feedings of whole formulated diets (30% starch inclusion) and a glucose control. Fasted dogs (n=8) and cats (n=8) were fed 1 g available carbohydrate per kg bodyweight (BW) of each diet or a glucose control (15% w/v solution). Values are mean ± SEM

Increases in GI were seen when the starch sources were formulated and extruded as whole diets. In dogs, GI for each individual starch versus the same starch in a whole diet was not significantly different. In contrast, for cats, the GI values for pulse starches significantly increased when formulated into whole, extruded diets compared to the dry-processed starches alone (pea $p = 0.028$; lentil $p = 0.048$; faba bean $p = 0.022$). Species comparisons for whole diet effects on postprandial plasma glucose showed significant species differences in peak plasma glucose for all four diets, modified cornstarch ($p=0.0005$), pea ($p=0.001$), faba bean ($p=0.005$) and lentil ($p=0.0005$), with all cat values significantly lower compared to dogs (Table 3.5). For time to peak glucose comparisons between species, only the glucose control produced a significantly longer postprandial time to glucose peak for cats ($p=0.0005$) compared to dogs. This was also seen when comparing the postprandial plasma glucose AUC results, only the glucose control produced a significantly higher result in the cats compared to the dogs. With all four of the diets, we saw no species differences in postprandial time to peak and postprandial plasma glucose AUC, despite a trend for cat AUC values to be higher than the dog results.

Postprandial plasma insulin results after single feedings of the glucose control or whole diets are summarized in Table 3.6, with time courses shown in Figure 3.2 for both species. For the dogs, the postprandial plasma insulin response after feeding the glucose control produced the highest peak, which was significantly higher than that of all test diets. In dogs, the modified cornstarch diet produced the next highest postprandial insulin response but was significantly different only from that of the lentil diet. The modified cornstarch diet produced the highest postprandial insulin AUC response in dogs, surprisingly numerically higher than the response to the glucose control, but no significant differences in postprandial insulin AUC were seen among

treatments. The dog insulin postprandial AUC values for the diets follow the same trend as the glucose GI results with a rank order of: modified cornstarch diet > pea diet > faba bean diet > lentil diet.

Cat plasma insulin results showed no significant differences among the control and diets for the time to peak insulin (min) and postprandial insulin AUC (pmol/L min). Peak plasma insulin was significantly higher for the glucose control, but only from the faba bean and lentil diets. Despite no significant differences, the glucose control produced the highest postprandial insulin AUC followed by modified cornstarch diet > pea diet > faba bean diet > lentil diet. The glucose control and the modified cornstarch diet postprandial insulin AUC follow the same trend as the glucose GI postprandial responses. The pulse starch diets produced both postprandial insulin and glucose responses lower than the modified cornstarch diet. Despite no significant differences, a trend was observed with the pulse starch diets producing lower responses than the modified cornstarch diet.

Whole diet postprandial insulin species comparisons showed significantly higher postprandial glucose peaks in the glucose control and three of the diets for dogs compared to cats. Glucose ($p=0.002$), modified cornstarch diet ($p=0.002$), faba bean starch diet ($p=0.011$), and lentil starch diet ($p=0.012$) were all significantly higher in dogs compared to cats. When looking at postprandial time to peak insulin values only the glucose control produced a significantly different value between species with cats having significantly longer time to insulin peak than dogs ($p=0.019$). The postprandial insulin AUC values showed no significant differences between species for either the glucose control or any of the four diets.

3.3.3. Methylglyoxal

Plasma methylglyoxal (MG) results are shown in Figure 3.3 for the single starch feedings and Figure 3.4 for the whole diet single feedings in both dogs and cats. Due to 60 minutes being intermediate between the peak plasma glucose response for glucose and all starches in both species, MG results are reported as a percent change from pre-feeding to 60 minutes postprandial. Because I hypothesized that high GI foods and higher hyperglycemia would produce the highest levels of MG, the order of data shown from left to right in Figure 3.3 and Figure 3.4 follows the order of GI values obtained in that same species, with highest GI value starch on the left to lowest on the right. In general, in dogs, the magnitude of increase in postprandial MG was observed to follow the rank order of GI values obtained. In dogs, the postprandial change in MG after feeding the glucose control produced a significantly higher increase compared to all three pulse starches, modified cornstarch and potato starch. In contrast, the postprandial change in plasma MG in dogs was not different between the glucose control versus the non-pulse starches from tapioca starch, white wheat flour, rice flour or unmodified cornstarch. In fact, plasma MG levels in dogs did not increase or may have even decreased at 60 min postprandial after a single feeding of all pulse starches (Figure 3.3).

The order of starches shown in Figure 3.3 for cats follows a different order than that of dogs since the rank order of GI values differed between species. In cats, postprandial plasma MG response to feeding the glucose control produced the highest increase but was significantly different from MG responses to feeding rice flour ($p=0.037$), potato starch ($p=0.01$), unmodified cornstarch ($p=0.015$), tapioca starch ($p=0.003$) and lentil starch ($p=0.015$). Most interesting was the observation that postprandial plasma MG levels decreased below fasting levels at 60 minutes postprandial in cats after feeding most starch sources, even to starches that

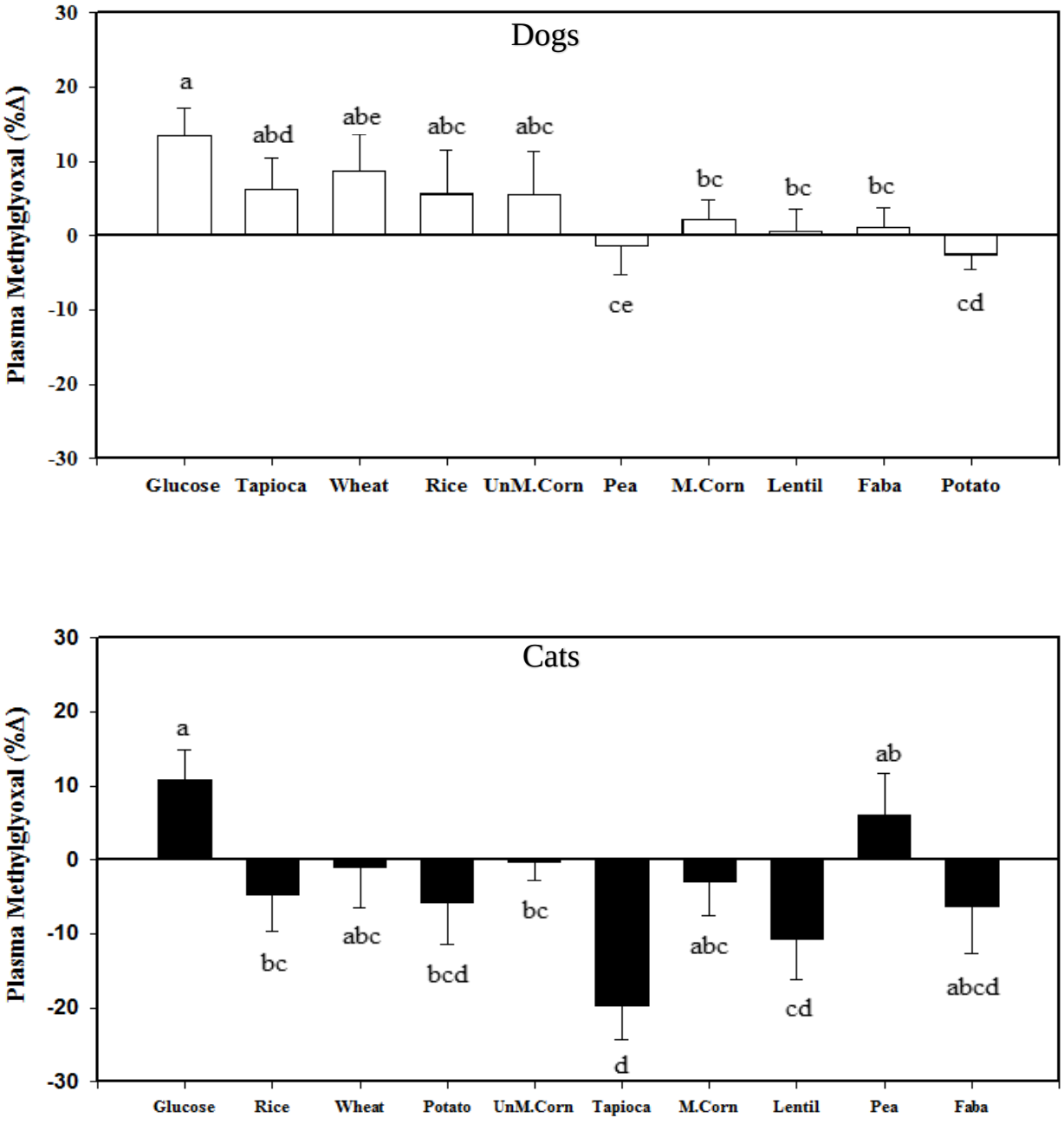

Figure 3.3 Plasma methylglyoxal levels as a percent change from time 0 (pre-feeding in fasted animals) to 60 minutes postprandial following single feedings of starches (1g available carbohydrate/kg bodyweight) or a glucose control (15% w/v solution; 1 g/kg). Results are shown for dogs n=8, and cats n=8. One-way repeated measures ANOVA followed by a least square difference post hoc test was used to determine significant differences ($p<0.05$) among the groups. Groups without a common letter differ from each other. M. Corn = modified cornstarch, UnM Corn = unmodified cornstarch.

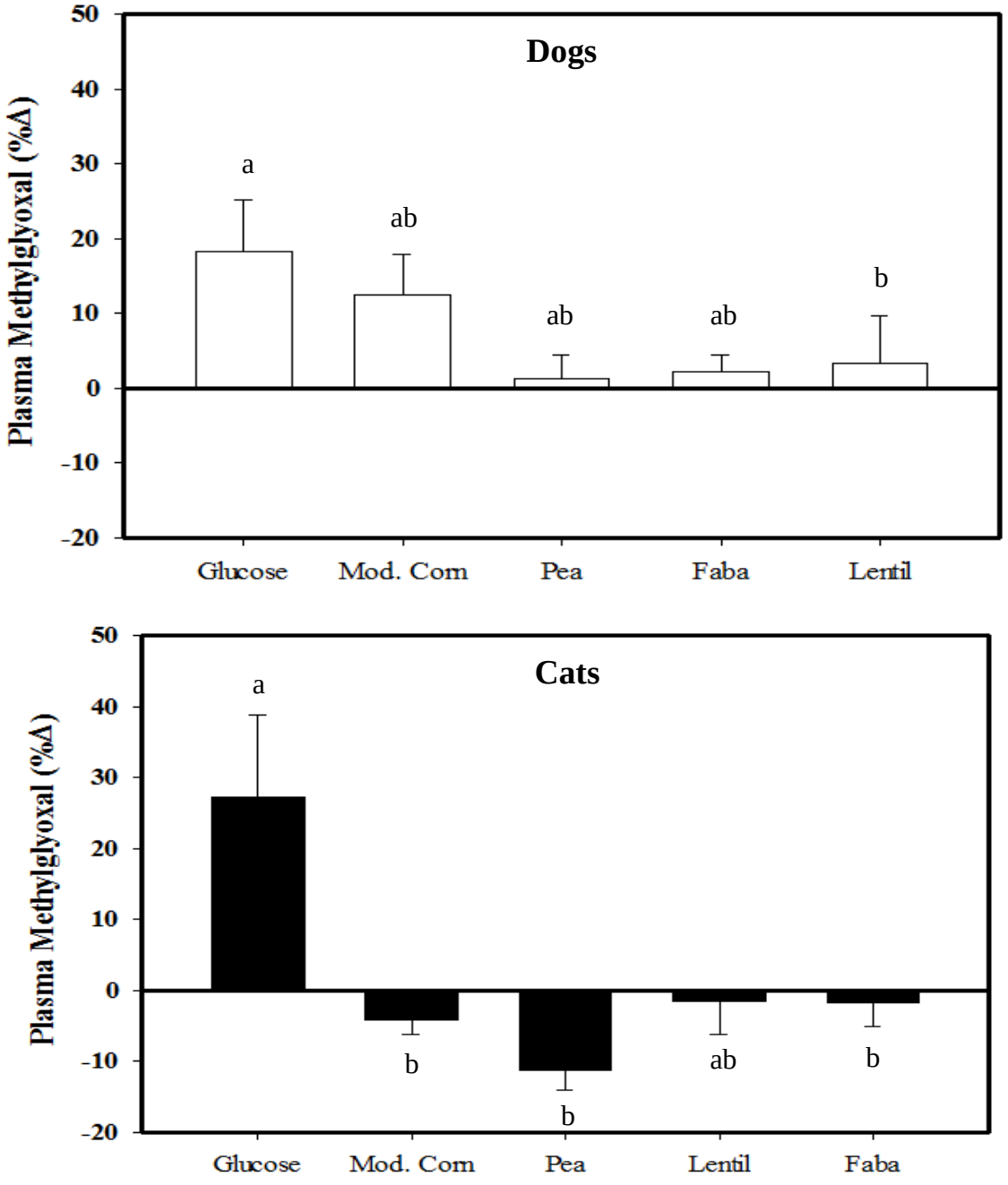

Figure 3.4 Plasma methylglyoxal levels as a percent change from time 0 (pre-feeding in fasted animals) to 60 minutes postprandial following single feedings of formulated diets (30% starch inclusion) or a glucose control (15% w/v solution) at 1g/kg. Results are shown in decreasing GI order for dogs n=8, and cats n=8. One-way repeated measures ANOVA followed by a least square difference post hoc test was used to determine significant differences ($p<0.05$) among the groups. Groups without a common letter differ from each other. Mod. Corn = modified cornstarch.

produced higher GI values in cats. In fact, the magnitude of change in postprandial plasma MG showed little relationship to the rank order of GI values in cats. The only other treatment that showed an increase in postprandial plasma MG levels in cats was pea starch, which was significantly different from both tapioca starch ($p=0.016$) and lentil starch ($p=0.044$).

Basal (pre-feeding) plasma MG levels were 0.699 ± 0.011 μM in dogs and 0.655 ± 0.013 μM in cats, indicating similar ($p=0.187$) fasting levels and basal production of MG in these species. Comparing postprandial plasma MG responses between dogs and cats, there were surprisingly consistent findings between species after feeding pure glucose (Figure 3.3). However, significant species differences were observed for tapioca starch ($p=0.001$). No other significant differences were seen between species for the other starches, despite most of the cat MG levels decreasing postprandially.

Postprandial changes in plasma MG levels after single feedings of whole diets compared to the glucose control are shown in Figure 3.4. In both species, similar to the previous experiment with purified starches, there was an increase in postprandial plasma MG levels following single feedings of the glucose control. In dogs, there was a general tendency for plasma MG to increase at 60 minutes postprandial, but of a lesser magnitude compared to the MG change elicited by the glucose control. Of the whole diets, the diet with modified cornstarch produced the numerically highest postprandial increase in plasma MG in dogs, but no postprandial plasma MG changes were significantly different from each other. Moreover, the postprandial change in plasma MG after feeding the diets tended to be lower than that of the glucose control in dogs, but only the postprandial plasma MG response to the lentil diet was significantly lower than the glucose control (Figure 3.4). In contrast, in cats, plasma MG levels decreased at 60 min postprandial from pre-feeding levels in response to feeding all four whole

diets, with responses to modified cornstarch, pea and faba bean diets significantly lower than the glucose control (Figure 3.4). When comparing species results, the only diet that produced significantly different results was the modified cornstarch diet (p=0.013). None of the other diet results were significantly different between species despite the cat results showing postprandial decreases where as the dogs showed postprandial increases.

3.3.4. Cardiovascular Parameters

Cardiovascular parameters including systolic and diastolic blood pressures (mm Hg), and pulse rate (bpm) were measured pre-feeding and at 60 minutes postprandial following each treatment in both cats and dogs. Results are shown in Tables 3.7 and 3.8 for single feedings of starches, while Tables 3.9 and 3.10 show results for single feedings of whole diets in dogs and cats, respectively. In dogs, systolic blood pressure tended to increase at 60 minutes postprandial compared to pre-feeding, but achieved significance only following consumption of tapioca starch ($p = 0.048$; Table 3.7). In contrast, in dogs, diastolic pressure stayed the same or tended to decrease at 60 minutes postprandial compared to pre-feeding, achieving significance following rice flour consumption ($p = 0.036$). Also, in dogs, a tendency for pulse rate to stay the same or decrease was observed at 60 minutes postprandial compared to pre-feeding but achieved significance after only unmodified cornstarch ($p = 0.001$) or modified cornstarch ($p = 0.028$) consumption.

Similar to what was observed in dogs, cat systolic blood pressure tended to increase at 60 minutes postprandial compared to pre-feeding but only achieved statistical significance ($p = 0.026$; Table 3.8) following consumption of pea starch. In contrast, diastolic blood pressure in cats showed no clear tendency and no significant changes at 60 minutes postprandial compared to pre-feeding after consumption of glucose control or any starch.

Table 3.7 Cardiovascular parameters in dogs following single feedings of glucose (control) and starches at times 0 (pre-feeding) and 60 minutes postprandial. Systolic and diastolic blood pressures (mmHg) and pulse rate (bpm) were measured using high-definition oscillometry.

Treatment	Time	Systolic	Diastolic	Pulse Rate
Glucose	0	135±3	64±2	81±5
	60	143±3	66±2	81±3
Tapioca	0	132±2	73±1	78±5
	60	137±3*	68±2	69±5
Wheat	0	134±4	70±2	75±4
	60	139±4	71±2	69±6
Rice	0	131±2	71±2	77±4
	60	135±2	66±1*	71±3
Corn	0	132±3	64±3	78±3
	60	139±3	62±2	66±3*
Pea	0	131±3	71±2	80±2
	60	136±2	70±2	76±4
Lentil	0	132±2	70±1	76±3
	60	137±3	71±1	75±4
Faba bean	0	138±4	68±2	78±3
	60	134±3	68±2	79±4
Potato	0	130±3	68±2	78±4
	60	135±3	68±2	73±2
Modified Corn	0	141±2	71±3	77±5
	60	142±2	69±1	66±3*

Results are shown in decreasing glycemic index order starting after the glucose control. Values are means ± SEMs, n = 8, in duplicate for glucose. Time 60 values with a * indicate a significant difference from time 0 within a treatment; $p < 0.05$, a paired t-test was used to determine differences among related samples within a treatment.

Table 3.8 Cardiovascular parameters in cats following single feedings of glucose (control) and starches at times 0 (pre-feeding) and 60 minutes postprandial. Systolic and diastolic blood pressures (mmHg) and pulse rate (bpm) were measured using high-definition oscillometry.

Treatment	Time	Systolic	Diastolic	Pulse Rate
Glucose	0	144±6	69±3	171±12
	60	155±2	73±1	200±12*
Rice	0	144±5	74±4	183±14
	60	146±5	69±3	198±14
Wheat	0	141±5	72±2	186±14
	60	151±7	73±4	200±14
Potato	0	146±4	74±2	182±12
	60	148±5	71±3	201±16*
Corn	0	140±5	74±3	158±11
	60	147±5	74±4	160±11
Tapioca	0	138±6	70±5	173±11
	60	146±4	71±3	206±19*
Lentil	0	145±4	70±2	182±12
	60	143±5	68±2	205±14*
Faba bean	0	149±4	72±3	182±11
	60	152±6	74±3	200±15*
Pea	0	136±4	71±2	178±11
	60	150±5*	72±3	202±9*
Modified Corn	0	141±4	71±2	186±13
	60	150±5	71±4	203±12

Results are shown in decreasing glycemic index order starting after the glucose control. Values are means ± SEMs, n = 8, in duplicate for glucose. Time 60 values with a * indicate a significant difference from time 0 within a treatment; $p < 0.05$, a paired t-test was used to determine differences among related samples within a treatment.

Table 3.9 Cardiovascular parameters in dogs following single feedings of glucose (control) and whole formulated diets at times 0 (pre-feeding) and 60 minutes postprandial. Systolic and diastolic blood pressures (mmHg) and pulse rate (bpm) were measured using high-definition oscillometry.

Treatment	Time	Systolic	Diastolic	Pulse Rate
Glucose	0	135±4	64±2	81±7
	60	143±5	66±2	81±3
Corn Diet	0	133±5	63±1	73±4
	60	140±4	63±2	75±2
Pea Diet	0	131±4	66±3	76±4
	60	132±2	64±2	73±4
Faba bean Diet	0	135±3	62±3	71±4
	60	139±5	61±2	74±4
Lentil Diet	0	137±5	60±2	74±4
	60	139±5	66±2*	76±1

Results are shown in decreasing glycemic index order starting after the glucose control. Values are means ± SEMs, n = 8, in duplicate for glucose. Values in a row with superscripts without a common letter differ; $P < 0.5$, one-way ANOVA with LSD post hoc test.

Table 3.10 Cardiovascular parameters in cats following single feedings of glucose (control) and whole formulated diets at times 0 (pre-feeding) and 60 minutes postprandial. Systolic and diastolic blood pressures (mmHg) and pulse rate (bpm) were measured using high-definition oscillometry.

Treatment	Time	Systolic	Diastolic	Pulse Rate
Glucose	0	140±4	70±3	163±12
	60	144±4	69±2	166±11
Corn Diet	0	144±5	71±3	160±9
	60	138±5	68±3	175±11*
Pea Diet	0	146±5	71±3	158±11
	60	142±6	71±3	183±11*
Faba bean Diet	0	148±5	71±3	157±11
	60	145±3	70±2	179±13*
Lentil Diet	0	144±4	71±3	151±10
	60	146±6	72±2	174±12*

Results are shown in decreasing glycemic index order starting after the glucose control. Values are means ± SEMs, n = 8, in duplicate for glucose. Time 60 values with a * indicate a significant difference from time 0 within a treatment; $p < 0.05$, a paired t-test was used to determine differences among related samples within a treatment.

Most interesting was the observation that cat pulse rate tended to increase at 60 minutes postprandial compared to pre-feeding. This postprandial increase in pulse rate was quite consistent and significantly different in cats following consumption of the glucose control ($p < 0.0005$), pea ($p = 0.001$), potato ($p = 0.005$), tapioca ($p = 0.02$), lentil ($p = 0.013$) and faba bean ($p = 0.007$) starches.

Results from the whole diet single feedings showed that in dogs (Table 3.9) there was a significant increase in diastolic pressure following consumption of lentil diet ($p = 0.002$), whereas in cats (Table 3.10) there was a significant increase in pulse rate following consumption of all of the diets (modified cornstarch $p = 0.002$; pea $p = 0.037$; faba bean $p = 0.001$; lentil $p = 0.002$), but not glucose. Systolic pressure and pulse rate showed no trends or significant changes in dogs at 60 minutes postprandial following consumption of glucose and all four diets, where as in cats systolic and diastolic pressures showed no trends or significant changes at 60 minutes postprandial following consumption of glucose or the diets.

3.4. Discussion

The present study was done to compare postprandial glycemic, insulinemic, MG and cardiovascular responses to different carbohydrate sources in an omnivorous species (dogs) versus a carnivorous species (cats) following single feedings of purified starches or whole formulated diets containing different starch sources. Pulse starches, with their low GI in humans (Foster-Powell et al. 1995) provide health benefits when consumed regularly. The most important findings of this study are that cats produced lower glycemic responses to purified starches and complete diets than the dogs, and had decreases in postprandial MG, which is opposite to what I hypothesized. The results in the dogs followed my hypotheses in that the pulse starches produced lower glycemic and insulinemic responses, compared to high GI starches,

following single feedings of the pure starches and the diets, with a clear trend between GI and postprandial MG production. In contrast, in cats, although pulses did produce lower glycemic responses compared to the other starches, the insulin responses were higher than what were expected and there was no clear link between GI and feline postprandial MG production.

3.4.1. Available Carbohydrate

Available carbohydrate is 'that fraction of carbohydrate that can be digested by human enzymes, is absorbed and enters into intermediary metabolism' as defined by the Food and Agriculture Organization (2003). Available carbohydrate does not include resistant starch or dietary fibre (FAO. 2003). Starch can be processed by either dry or wet milling of whole seeds or grains. Dry processing involves air classification to separate the starch fraction, whereas wet processing involves soaking the seeds and an acid-base treatment. For this thesis, dry-processed pulse starches were used. The *in vitro* available carbohydrate assays showed that unmodified corn, rice flour, tapioca starch and potato starch contained the highest amounts of available carbohydrate at >97%. This is consistent with the literature where unmodified corn, rice and potato starches are reported to contain >97% non-resistant starch (Murray et al. 1999). White wheat flour has been reported to contain between 76.3-79.3% available carbohydrate (Marinangeli & Jones. 2011; Abebe et al. 2015) which is similar to our results of 81.7% available carbohydrate. Pulse starches, likely all of which was wet processed, have been reported to contain between 62-75% total available carbohydrate (Berrios et al. 2010; Ambigaipalan et al. 2011), but is still consistent with our findings of between 71-76 g available carbohydrate/100 g starch of dry-processed pulse starches.

Previous results from our group reported *in vitro* available carbohydrate values of 88.9 ± 0.8 for whole rice, 71.4 ± 0.6 for whole corn and 50.8 ± 1 for whole peas (Adolphe et al. 2012).

Processing and purification of the starches can lead to changes in *in vitro* and *in vivo* digestibility by changing the starch granule structure, along with removing protein or lipid that is found in native, unprocessed pulses. The higher protein and resistant starch content of whole peas used in the previous study in our lab compared to the dry-processed pea starch used in this thesis are consistent with known changes in purity and corresponding changes in availability. Similarly, the lower values reported in Adolphe et al. (2012) for whole white rice is likely explained by the fact that even white rice retains some protein, lipid and resistant starch, thereby lowering the available carbohydrate compared to the highly purified wet-processed rice flour used in this thesis. The modified cornstarch used in this thesis is covalently modified with hydroxypropyl substituted cross-linked groups to improve resistance to high temperature, low pH and shear degradation, conditions often encountered in food processing (Tate & Lyle. 2016). In this thesis, the modified cornstarch had an available carbohydrate content of 65% which is comparable to what is reported in pulse starches. Chung et al. (2008b) has shown that modification of cornstarch, such as with hydroxypropyl, increases the resistant starch content (not included in available carbohydrate content) and lowers the slowly digestible starch content (the major fraction available for digestion). This is confirmed by our findings which showed a decrease from 100% available carbohydrate content for the unmodified cornstarch to 65.95% for the modified cornstarch. Thus, this formed the rationale for using this lower starch availability cornstarch in this thesis. The goal was to use a cereal starch that had similar starch availability as the pulse starches, so that differences I observed in glycemic responses, MG production and cardiovascular effects were more related to cereal versus pulse properties other than available starch.

3.4.2. Postprandial Glucose Responses

Oral dosing is the standard method in humans when determining GI to test a foodstuff. Although oral glucose dosing has been shown to be more variable among humans (Church. 1980), it is more relevant for nutrition and diet studies because starch will undergo the same metabolism, exposure to digestive enzymes and glucose transport when food is ingested. The methodology employed in this thesis using glucose as a control is identical to an oral glucose tolerance test (OGTT) used to assess insulin sensitivity and to test for diabetes in humans and animals. Differences in glucose metabolism between dogs and cats has been reported in previous studies. The time to peak plasma glucose was significantly different between species when the glucose control treatment or OGTT was performed. In dogs, the time to peak plasma glucose was 35 ± 2 (min), whereas the cats were significantly longer at 57 ± 2 (min) which follows trends in previous studies in dogs (Church. 1980; Adolphe et al. 2012 & 2015) whereas in cats, Hoenig et al. (2010) reports peaks similar to dogs. Oral glucose tolerance test data in cats is sparse. Most often intravenous GTT is used (Theiss et al. 2004; Appleton et al. 2004; Verbrugghe et al. 2009; Farrow et al. 2013). This difference in blood glucose appearance could be due to dogs having increased function or expression of sodium/glucose cotransporter-1 in the small intestine (Batchelor, et al. 2011) which would result in glucose passing into the bloodstream at an increased rate. However, surprisingly, the peak blood glucose levels in the dogs and cats following an OGTT are not significantly different from each other at 6.8 ± 0.3 and 6.9 ± 0.5 mmol/L respectively, disagreeing with my hypothesis. Moreover, one of the most interesting findings in this thesis is that the plasma glucose peak levels in cats all tended to be lower than the dogs following single feedings of each starch. For example, following rice flour, dog peak plasma glucose was 5.5 ± 0.1 mmol/L, while that in cats was 4.8 ± 0.2 mmol/L. Since peak OGTT glucose levels are similar between species, lower glucose transporter function in cats is

not an explanation for lower glucose responses to starches and instead, may relate to lower levels of amylase or other starch-digesting enzymes. Other studies have looked at different carbohydrate sources in cat food (Kienzle. 1994a, Appleton et al. 2004, de-Oliveira et al. 2008), but have only looked at the glucose responses after feeding the starches in whole formulated diets, not the starches alone. These results showed that cats may be able to handle higher amounts of carbohydrates than originally thought. Moreover, unlike humans and dogs, in cats, carbohydrates may not be the main nutrient responsible for postprandial changes in blood glucose. In dogs it has been reported that the main determinant of postprandial glucose is the amount of exogenous carbohydrate or starch ingested in diets (Nguyen et al. 1998). If starches are highly digestible in cats (data shown in chapter 4) and the glucose is not going into their blood, I examined some fecal samples to determine if they were excreting the glucose and from this I found that the glucose was not being excreted in the feces (results not shown). Previous studies have shown minimal traces of glucose being excreted in urine following feedings of potato or cornstarch diets (Kienzle. 1994a). Perhaps, the glucose is being utilized as a substrate for fermentation in the hindgut, but this was not assessed.

In both species, single feedings of rice flour and unmodified cornstarch produced the highest postprandial plasma glucose peaks. This is expected considering both types of starch have high amounts of rapidly digestible starch (RDS) (Murray et al. 1999). All pulse starches (pea, lentil and faba bean) produced peaks in plasma glucose that were significantly lower than rice flour in both species, consistent with the higher amounts of resistant starch (RS) and slowly digestible starch (SDS) content of pulse starches (Cummings & Englyst. 1995). Pulses also contain higher levels of amylose compared to amylopectin (Singh et al. 2010) both of which are composed of glucose units, although amylopectin, which is branched, is more susceptible to enzymatic

breakdown into glucose than amylose, which is linear. Potato starch, which has a type B granule that is resistant to breakdown by pancreatic amylase, produced a plasma glucose peak similar to the pulse starches in dogs, but the lowest peak in cats. Similar results were shown by Gunawardena et al. (2010) in pigs, where potato produced the lowest postprandial glucose and insulin blood levels compared to corn, tapioca, wheat, pea and faba bean starch sources. While potato starch would be beneficial in terms of glucose and insulin control when included in pet foods, it has also been shown to have decreased ileal nutrient digestibility compared to diets containing corn or wheat (Murray et al. 1999). White wheat flour produced intermediate plasma glucose levels when compared to other starch sources, suggesting this would be a better option than rice flour or cornstarch for pet foods. Wheat has been reported to contain less RDS and more SDS than other cereal starch sources such as corn and rice (Murray et al. 1999).

This thesis found that in dogs, postprandial time to peak plasma glucose was significantly quicker after feeding the glucose control or rice flour compared to pulse starches or tapioca starch. Similar results were shown by Carciofi et al. (2008), in that pea (55±5 min) and lentil (87 ± 20) starch-based diets produced the longest time to peak blood glucose compared to corn and rice (both 15 ± 0.1). Rice flour and glucose in both species in this thesis produced similar times to postprandial peak plasma glucose (± 1 min of each other), showing that rice flour is rapidly broken down into glucose and rapidly absorbed. In this thesis, white wheat flour was shown in cats to take the longest to produce peak plasma glucose, which was not significantly different from lentil starch, and potato starch. Interestingly, the other two pulse starches (pea and faba bean) produced quicker times to peak plasma glucose than the glucose control, which was unexpected considering their high content of RS and SDS. Despite rapid time to peak plasma

glucose compared to the glucose control, the glycemic response remained low to pea and faba bean starches in both species since peak levels and AUC were low.

For the single feedings of the purified starch sources I used an unmodified cornstarch that would typically be used in diet formulations, while for the whole diet I used a modified cornstarch. This was done to examine if using a hydroxypropyl cross linked modified cornstarch would produce glucose and insulin responses which closely resembled results from the pulse starches, and if so would this then be a beneficial starch to use in pet food. Modified starches are made to enhance certain physiochemical properties such as thickening and gelling which is one of the main reasons they are used in food formulations. Modifying starches can increase the amount of resistant starch content which is desirable as it acts like dietary fibre which has been associated with health benefits such as bowel health maintenance, weight control, and prevention of diabetes and cardiovascular health (Champ et al. 2003). Chung et al. (2008b) has shown that hydroxypropyl cornstarch has an increased percentage of resistant starch compared to unmodified (34.2% vs 11.9%) therefore this decreases the estimated *in vitro* GI from 75.3 to 70.5 in the granular state and 93.5 to 86.4 in the gelatinized state.

When comparing results between the two species we can see that when fed the pure glucose control, cats (58 &180 min) take double the time of dogs (35 & 90 min) to reach postprandial plasma glucose peak and return to baseline values. Other studies have also reported a prolonged time for glucose elimination in cats compared to dogs and humans (Bouchard & Sunvold. 2000; Appleton et al. 2004). These results could be due to specific feline adaptations due to their carnivorous diet. Cats are reported to be in a constant state of gluconeogenesis which contributes an endogenous source of glucose to any exogenous carbohydrate consumed (MacDonald et al. 1984). Cats are also reported to have delayed gastric emptying with mean times reported of 24 h

when fed their full daily energy requirement (Coradini et al. 2006) which can also contribute to delayed time to peak blood glucose and delayed return to baseline. Other studies report elevated blood glucose levels 8+ hours following a high starch meal in cats (Farrow et al. 2012; Hewson-Hughes et al. 2011; Appleton et al. 2004). To determine if this was in fact due to the starch component of the diet an additional study was performed in which blood glucose responses were evaluated over 12 hours to single feedings of glucose, rice flour, and pea starch (Appendix A.1). These results showed postprandial blood glucose levels were back to baseline by three hours suggesting that it is not the starch content causing this delayed increase in blood glucose levels, which agreed with Mori et al. (2016), which shows no blood glucose increases following six hours. This reported extended elevation in blood glucose levels may be explained by the other macronutrients in the diet, such as protein and fat, being utilized as an energy source.

3.4.3. Glycemic Index and Area Under the Curve

Previous work done in our lab reported AUC and GI values for whole white rice and corn in beagles (Adolphe et al. 2012), agreeing well with values for starch sources from these same foods in this thesis in a different cohort of beagles. However, the pea starch GI value obtained in beagles in this thesis were higher than the GI of 29 for whole peas reported by Adolphe et al. (2012). This higher pea starch GI is likely due to the loss of structure, reduced fibre and reduced protein content during dry processing, but could also be due to differences in the variety of pea used (yellow peas used for both, but varieties not known). The GI of pulses varies depending on type of pulse, how it was processed and *in vitro* vs *in vivo* analysis. Whole pulse GI in humans has been reported to range between 30-60 (Foster-Powell et al. 1995; Foster-Powell et al. 2002) where as *in vitro* GI determined for pea and lentil flours were predicted to be between 41 and 48 (Chung et al. 2008a). A later study by Chung et al. (2012) also showed differences *in vitro*

digestibility between pea flour and pea starch, with the estimated GI (eGI) of pea flour around 37 and the eGI of pea starch (wet processed) around 70, agreeing well with observed differences in dog GI values for pea starch in this thesis versus whole peas in previous studies from our lab (Adolphe et al. 2012). Estimated GI is an *in vitro* estimation of the glycemic response which uses the % of starch hydrolysis (Goni et al. 1997). In this thesis, no significant differences were observed among different diets for the postprandial AUC and GI values in dogs, which is similar to results reported by Carciofi et al. (2008) for postprandial AUC for corn, rice and peas. Previous studies have reported corn to produce significantly lower glucose levels than rice (Bouchard & Sunvold. 1999) which is not what this study, Adolphe et al. (2012) or Carciofi et al. (2008) found.

To our knowledge, there are no previous studies reporting GI values for starch sources in cats. Glycemic index values and postprandial AUC values in cats showed that rice flour and white wheat flour produce the highest results, whereas pulse starches produced the lowest GI and postprandial AUC values with negligible responses. Potato starch produced one of the higher AUC and GI values in cats, which was not seen in dogs. This may be due to the longer plasma glucose time to peak seen in cats compared to dogs, which results in blood glucose levels being elevated above baseline for longer periods of time and contribute to a higher postprandial AUC and GI. Potato starch is the only treatment that produced a higher GI value in cats versus dogs. This is an unexpected result and is contrary to the hypothesis that cat GI responses would be higher than in dogs. This may be due to the fact that cats, being strict carnivores, have been reported to use proteins as a preferred source of energy (Keinzle. 1993).

In this thesis, increases in GI were seen when the starch sources were formulated and extruded as whole diets. These results not only showed the effects of processing, but major

species differences in patterns of responses to individual starch sources versus whole diets. As mentioned previously, this increase in GI in both species could be due to effects of processing. However, particularly with cats, effects of other macronutrients such as protein and fats in the whole diets may have had profound effects on glycemic responses.

3.4.4. Postprandial Insulin Responses

Insulin results in dogs showed some interesting yet contradictory results to the glucose responses. Tapioca starch produced the lowest postprandial peak insulin, although only significantly different from glucose, white wheat flour and rice flour, yet produced the highest AUC and GI in dogs, a result that is unusual. White wheat flour, rice flour and potato starch showed results that were expected with higher AUC values for both glucose and insulin in white wheat flour and rice flour and the lowest AUC values for potato starch. Carciofi et al. (2008) looked at insulin responses to different starch sources in dog food and found that the rice diet produced the highest peak insulin values compared to corn, lentil and pea diets, but was only significantly different from the lentil starch diet. Our results showed rice flour produced significantly higher insulin peaks than lentil, pea and unmodified cornstarches. Very few studies to our knowledge have evaluated the effects of the separate components of the diet alone. Results previously reported in our lab by Adolphe et al. (2012) found that peas produced the lowest responses compared to corn, rice and barley, although not significantly different from each other. However, this study used whole dried carbohydrate source whereas I only used the purified starch fraction in this study.

Insulin in cats from the single starch feedings showed some interesting results which warrant further research on effects of carbohydrates in obligate carnivores. Following feeding of the purified starch sources in the cats, the pulses produced the fastest time to peak, and moderate

peaks even though these starches produced the lowest GI results. These results suggest that there is something else in the pulse starches stimulating insulin release in cats. Although the starches are purified, the protein content in the pulse starches are still ~15%, could be exerting an insulinotropic effect. A study done by Martin et al. (2014) showed that cats produce a significant insulin response to arginine infusion, which peaked within the first 10 minutes. Cats were also found to produce a mild response to other amino acids such as alanine and leucine, which peaked within the first 20-30 minutes following infusion. This study was done in obese cats, while an earlier study by Kitamura et al. (1999) showed similar results following an arginine infusion but in healthy non-obese cats, with rapid insulin peaks within the first five minutes and returning to baseline within 30 minutes. This shows that the cat pancreas is highly responsive to amino acids and has been suggested to be more responsive to amino acids than glucose (Kitamura et al. 1999). Comparing the composition of corn, rice, wheat, pea and faba bean, the arginine content (g/kg) in peas and faba beans is highest at 19.0 ± 3.3 and 21.0 ± 3.5 followed by rice 7.2 ± 1.4, wheat 6.6 ± 1.3 and corn 3.8 ± 0.6 (Stein et al. 2016). Alternatively, Verbrugghe et al. (2010) has suggested that the carbohydrate effect on insulin sensitivity is a U-shaped curve, in that too much or too little carbohydrate can have negative impacts on insulin sensitivity potentially leading to insulin resistance.

Whole diet extrusion processing has been shown previously in this lab to increase GI when comparing unprocessed individual ingredients to diets that include the ingredient (Adolphe et al. 2012; Adolphe et al. 2015). Previous results showed that following extrusion, the GI of peas went from 29 ± 5 to 56 ± 12, although not significantly different in dogs. In this thesis, a similar effect of processing was seen following feedings of all three pulse starches and diets, and the cornstarch and diet, in both cats and dogs. However, the increase in GI following processing was

more pronounced in cats. Since these animals will be consuming whole diets and not just the starch over their lifetime this increase in GI from processing is important to consider when formulating diets. High GI diets could have negative long-term consequences on metabolic processes, weight control and cardiovascular parameters.

3.4.5. Postprandial Methylglyoxal Responses

Linking the methylglyoxal levels to the glycemic responses in both species shows interesting results. Previous work done in our lab (Adolphe et al. 2012), reported similar increases in postprandial MG levels in dogs following single feedings of simple (glucose) and complex (corn, rice and pea) carbohydrate sources. However, this previous study did not find differences among different complex carbohydrates on postprandial plasma MG levels in dogs, while work in this thesis found that pea starch produced significantly smaller magnitude postprandial plasma MG increases compared to tapioca starch in dogs. In cats, where MG and postprandial responses have not been previously reported, postprandial responses in plasma MG for all but one of the starch sources decreased, instead of increasing. Only pea starch and the glucose control produced postprandial increases in plasma MG levels in cats. Thus, when comparing the GI values and postprandial MG levels in dogs, there is a clear relationship. For dogs I can accept the hypothesis that starch sources with higher GI values will produce higher amounts of MG following a single feeding of starch. In contrast, in cats, this link between the GI and MG levels is not apparent and the hypothesis that higher GI starch sources will produce higher postprandial MG levels is rejected.

Looking at the results from the whole diet feedings there is a similar trend in the dogs with the postprandial MG levels following the same trend as the GI. The modified cornstarch diet produced the highest GI and also produced the highest postprandial increases in MG, although

not significantly different from the other diets. In the cats, again we see decreases in postprandial levels following acute feedings of the whole diets. MG levels following the pea diet decreased, whereas we saw an increase with the pea starch feeding which seem to contradict each other.

Blood levels of MG have previously been reported to be elevated in patients with both type 1 (Han et al. 2007) and 2 diabetes compared to nondiabetic patients (Wang et al. 2007), but these studies looked at baseline levels and not postprandial levels. One study comparing postprandial MG levels in diabetics to nondiabetics showed a 3.6 and 2.7-fold increase respectively following consumption of a standardized breakfast meal containing 65 g of carbohydrates (Boes et al. 1999). A study done in healthy male patients showed increases in postprandial levels following an oral glucose test (Masterjohn et al. 2012) which we can relate to the results of the dogs since both are healthy weight, nondiabetic omnivores. These are some of the few studies published that reports postprandial increases in MG levels in healthy individuals. Diet has been reported to play a role in AGE production, which can occur from MG interacting with various proteins (Jia et al. 2006). Krajcovicova-Kudlackova et al. (2002) reported that vegetarians had higher levels of plasma AGEs compared to people who consumed omnivorous diets and suggested the higher intake of fructose as the cause. Hipkiss (2005) suggested that the consumption of a carnivorous diet may play a beneficial role in protecting against carbonyl stress and AGE formation due to the intake of carnosine, a dipeptide only found in animal tissues. Cats may have developed, through their carnivorous diet, the ability to better utilize carnosine as a protective agent, as they would naturally consume higher levels of animal tissues. This may play a potential role as to why we see decreases in postprandial MG levels. Another protective mechanism to detoxify MG is through the glyoxalase system which uses reduced glutathione (GSH) to convert MG into D-lactate (Kalapos. 1999). GSH levels have been shown to be reduced following MG exposure in

mice (Ankrah et al. 1999) which could interfere with other GSH-dependent processes and cause oxidative stress and potential endothelial dysfunction. Healthy dogs and cats have been shown to have similar erythrocyte GSH levels (Viviano et al. 2009) so both have the potential for detoxifying MG. Further research is needed into the mechanism by which cats are able to decrease postprandial levels of MG, despite increasing levels of plasma glucose.

3.4.6. Postprandial Cardiovascular Responses

One of the interesting findings in this thesis is that systolic blood pressure tended to increase, albeit not always significantly, at 60 min postprandial after feeding glucose control or any of the starch sources in both dogs and cats. In dogs, increases in systolic pressure at 60 min postprandial almost achieved significance for glucose ($p = 0.067$) and white wheat flour ($p = 0.061$), with these carbohydrate sources also producing the highest AUC values and GI responses. In cats, pea starch was the only treatment to cause a significant postprandial increase in systolic pressure, which was coincidentally also the only complex carbohydrate to produce a postprandial increase in MG. In rats, MG has been shown to increase renin-angiotensin levels and therefore blood pressure (Dhar et al. 2014), but this was following a four-week continuous infusion of MG. Increases in systolic pressure were also noted following consumption of fructose treated water for 11 weeks in rats (Vasdev et al. 1998). Higher systolic pressure can also be caused in the short term by increased heart rate, a trend that is supported by the data in cats in this thesis, but not in dogs. Therefore, it is more likely that postprandial increases in MG are linked to higher systolic pressure through the well-known ability of MG to cause oxidative stress, resulting in inactivation of nitric oxide and endothelial dysfunction (Dhar et al. 2014). It is important to examine the cardiovascular effects of the whole diets in longer term feeding studies to gain a better understanding if pulse diets will have benefits in terms of cardiovascular health.

3.4.7. Conclusions

In conclusion, the results of this study showed that pulse starches are healthy alternative carbohydrate sources for pet foods based on low postprandial glycemic and insulinemic responses in both cats and dogs. To our knowledge, no other studies have compared metabolic responses to starch sources alone, only formulated in diets. This provided a better understanding of species *in vivo* differences to carbohydrate metabolism while minimizing confounding factors such as protein and fat digestion. Although species differences were observed in carbohydrate metabolism between cats and dogs, this does not mean that cats cannot efficiently utilize carbohydrates in their diets which we can see from our other results. However, there are species differences that need to be taken into consideration concerning how the type of carbohydrate affects starch metabolism, glycemic responses and MG production in dogs and cats. Other studies have examined postprandial metabolic responses in dogs and cats, but some did not report the type of starch they used, some continued to feed the animals during testing, and some used obese animals (Mori et al. 2016).

Pulses not only add low GI carbohydrates to the diet but protein as well, which is why they are consumed by humans as a meat protein substitute and increasing in popularity as an ingredient in pet foods. Pet foods tend to follow human health trends, many of which are not scientifically based. More pet food companies are producing food that is wheat free, grain free or 'gluten free', meaning that non-cereal carbohydrate sources (*i.e.* sources other than wheat, rice or corn) must instead be used. The results of this thesis showed that pulse starches are beneficial in both dogs and cats for lowering postprandial blood glucose responses. Therefore, pet foods produced with pulse starches can now include scientifically-based claims for marketing that their 'grain-free', pulse starch-containing food is a healthier choice.

4. EFFECTS OF LONG-TERM FEEDING OF WHOLE DIETS FORMULATED WITH 30% INCLUSION OF MODIFIED CORNSTARCH VERSUS PULSE STARCHES IN AN OMNIVORE COMPARED TO A CARNIVORE

This study is the second part of the overall research work looking at effects of pulse starches as an alternative carbohydrate source in dogs and cats. The first study was examining acute effects of the starch ingredients alone and formulated into diets, while this second study examines the long-term feeding of the whole diets in a crossover study design. This study will provide a better understanding of health effects following consumption of pulse diets compared to a cornstarch-based diet.

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Jennifer Briens did 100% of the animal work, data collection and data analysis for glucose, insulin, cardiovascular parameters, and oxidative stress as well as all writing of the manuscripts. Dr. Lynn Weber providing expertise and major editing of this thesis. Dr. Kaushik Desai contributed to methylglyoxal data collection by providing equipment, expertise and input for the HPLC work. Dr. Jennifer Adolphe contributed 100% to the diet formulations by providing us with balanced diets.

4.1. Introduction

A well-balanced diet consisting of carbohydrates, proteins, fats, vitamins and minerals plays an important role in maintaining overall health in not only humans, but companion animals as well. To determine the effects of different carbohydrates on glucose parameters, the GI methodology was designed to evaluate postprandial responses to different food sources in humans (Wolever et al. 1991). Pulses (peas, lentils, chickpeas and beans) have been shown in humans to produce low GI values (40-60) compared to other carbohydrates such as corn (90-100), but less is known about GI values for dogs and cats. Long-term consumption of low GI foods has been well documented to play a beneficial role in terms of weight control, inflammation, metabolic control and chronic disease prevention in humans (Jenkins et al. 2002; Pereira et al. 2014). When considering if the same is true for companion animals, there is still a lack of research to clearly indicate health benefits of long-term low GI food consumption, especially in cats.

Novel pet food ingredients and grain-free diets are becoming increasingly popular among pet food companies and consumers. The use of pulses as a pet food ingredient has risen in popularity, due to their appeal as a grain free alternative and known health benefits in humans (Pet Food Industry. 2017). Chickpea, peas, and lentils are the most popular options used in pet food formulations (Pet Food Industry. 2017). The use of this novel ingredient can expand the market for pulse crops, of which Canada represents 35% of total global market pulse production trade. Overall, Saskatchewan is a top Canadian producing province providing 90% of Canadian pulse exports (Saskatchewan Pulse Growers. Accessed May 2017).

Various nutritional studies have looked at carbohydrate content in both canine and feline diets. Digestibility of starch sources in formulated diets has been reported to be > 98% in dogs

for corn, rice, cassava flour, pea and lentil (Murray et al. 1999; Carciofi et al. 2008) whereas in cats, digestibility of starch in formulated diets is >95% for corn, rice, cassava flour, pea and lentil (de Oliveira et al. 2008) which is lower than dogs, but still highly digestible. It has previously been reported that the amount of digestible carbohydrate in the diet is the main determinant of postprandial glycemic responses in dogs (Nguyen et al. 1998; Elliott et al. 2012). Modified cornstarch has been shown to increase the amount of resistant starch *in vitro*, which is not digestible, but the same has not been determined *in vivo* in dogs or cats. Therefore, decreasing the amount of digestible carbohydrate can lower blood glucose levels and potentially be beneficial for managing glycemic excursions in diabetic or overweight dogs. The same does not appear to be true for cats (Verbrugghe et al. 2010), with both high and low carbohydrate diets negatively affecting insulin sensitivity. In humans, it is not only the amount of carbohydrate consumed, but the type of carbohydrate which effects glucose and insulin responses (Rebello et al. 2014). This has been shown to be the same for both dogs (Carciofi et al. 2008) and cats (de-Oliveira et al. 2008), but with these studies GI was not calculated. Therefore, I wanted to compare isoenergetic levels of both low and high GI carbohydrate sources in diets to determine long-term health effects and compare glycemic and insulinemic responses in an omnivorous species, dog, compared to the carnivorous domestic cat. I chose modified cornstarch as our cereal grain starch source to evaluate if the modification to the starch structure will result in a healthier cornstarch option, and more closely resemble the pulse starches (Chung et al. 2008a). The pulse starches I chose were pea, lentil, and faba bean to expand on previous studies in our lab using peas (Adolphe et al. 2012; Adolphe et al. 2015) and because these are becoming increasingly common ingredients in pet foods.

Hyperglycemia can lead to the production of toxic glucose metabolites, particularly methylglyoxal (MG) (Matafome et al. 2012) which have been shown to be increased in humans with chronic hyperglycemia (diabetes) (McLellan et al. 1994; Wang et al. 2007). MG can induce oxidative stress both directly and indirectly through the production of advanced glycation end products (AGEs) (Desai et al. 2010). Not only is MG increased in the plasma of diabetics, but it has been shown to be increased by 35% in obese patients compared to non-obese patients (Masania et al. 2016) and is also linked to the production of inflammatory biomarkers (Dhar et al. 2008). Therefore, chronic intake of high GI foods can lead to postprandial hyperglycemia and increases in MG, oxidative stress, and inflammation, which in turn can negatively affect nitric oxide, endothelial function (Sena et al. 2012) and cardiovascular health. I hypothesized that chronic feeding (six weeks) of the three pulse-based diets would improve glycemic control, increase insulin sensitivity, improve cardiovascular health, decrease postprandial levels of MG and decrease oxidative stress compared to after chronic feeding of the modified corn-based diet in both cats and dogs. These effects will be especially prominent in cats due to their carnivorous nature.

The purpose of this study was to first evaluate digestibility of the pulse starch diet versus the modified cornstarch diet, then determine the long-term health effects of consuming a diet with 30% inclusion of pulse starch (pea, lentil or faba bean starch) as the carbohydrate source versus 30% modified cornstarch using a six-week crossover feeding trial in dogs and cats. To evaluate the health effects, end-points analyzed at the end of each six-week feeding period were metabolic parameters (postprandial glucose and insulin responses after a glucose or test diet meal challenge), cardiovascular parameters (baseline and postprandial blood pressure and arterial

blood velocity), as well as postprandial changes in methylglyoxal and pre-feeding nitrotyrosine levels.

4.2. Materials and Methods

4.2.1. Animals

Beagle dogs (n=8, four males and four females; neutered/spayed; 3-4 years old) and mixed breed domestic cats (n=9, four males and five females; neutered/spayed; 3-4 years old) were obtained from a certified scientific breeder. The cats roamed freely during the day in the Animal Care Unit (ACU) but were housed individually in kennels during feeding and at night in the ACU at the Western College of Veterinary Medicine (WVCM). The dogs were group-housed in an adjacent dog kennel area during the day with access to outdoor runs but housed in individual kennels during feeding and at night. The dogs were walked daily, either on leash or off-leash in a dedicated fenced outdoor dog yard at the ACU. The dogs were fed two meals a day, once in the morning and once in the early evening, while the cats were fed one meal a day, in the early evening. This was kept standard throughout the entire experiment regardless of diet (experimental diets and commercial diet). Prior to feeding trials, animals were fed amounts based on previous husbandry experience and adjusted weekly for each individual animal to maintain ideal body condition scores. Average body condition scores were between 4-5 based on a 9-point scale (Laflamme. 1997a, 1997b). Blood samples were collected for analysis of plasma from each animal following each crossover period and sent to Prairie Diagnostic Centre, located in the Western College of Veterinary Medicine (Saskatoon, SK, Canada), for small animal blood chemistry panel, and complete blood count (CBC) analyses. Fecal quality was assessed qualitatively for noticeable changes but not scored. All animal handling and procedures were

conducted according to a protocol approved by the Animal Research Ethic Board at the University of Saskatchewan under the guidance of the Canadian Council on Animal Care.

4.2.1. Digestibility Testing

Digestibility test diets were formulated to meet daily nutrient and energy requirements of both dogs and cats (The Association of American Feed Control Officials. 2014). Four digestibility test diets to be used in both species were formulated using our test starches (modified corn, pea, lentil, and faba bean) at a 30% inclusion rate and used to feed both cats and dogs (see Table 4.1 for formulation). Diets were formulated using Concept 5 software (Creative Formulation Concepts, LLC, MD, USA) by Dr. Jennifer Adolphe, (Senior Nutritionist, Petcurean, Chilliwack, BC, Canada). To track the apparent digestibility of the diets, the indicator method was used as previously described in this lab (Adolphe. 2013; Association of American Feed Control Officials. 2009). Celite, an inert marker, was used as a tracer and added at an inclusion rate of 1% in the diets. The diets were extruded at the Saskatchewan Food Industry Development Centre located at the University of Saskatchewan (Saskatoon, SK, Canada), using a Clextral Evolum 32 twin screw extruder (Firminy, France) with a 24:1 length diameter ratio and a 2.88 mm die. Extrusion parameters were as follows: barrel zone temperatures from zone one to zone six were 50°C, 80°C (feeding section), 100°C (mixing section), 125°C, 130°C and 130°C (cooking section), screw speed (rpm) 371 for pea and lentil diets, 396 for faba bean and modified cornstarch diets, total moisture in extruder (%) 29.9 for pea diet, 27.2 for lentil diet, 35.4 for faba bean diet, and 27.5 for the modified cornstarch diet. The digestibility trial was tested using n=4 for dogs (four neutered males) and n=5 for cats (four neutered males; one spayed female).

Table 4.1 Diet formulations used for digestibility determination of test starches in both cats and dogs. Test diets were nutritionally complete with 30% inclusion of modified corn, pea, faba bean or lentil starch plus 1% Celite.

Ingredient	Corn Diet (%)	Pea Diet (%)	Faba bean Diet (%)	Lentil Diet (%)
Cornstarch	30.00	-	-	-
Pea Starch	-	30.00	-	-
Faba bean Starch	-	-	30.00	-
Lentil Starch	-	-	-	30.00
Chicken meal	42.7	23.96	17.18	22.34
Soy Protein concentrate	9.21	15.00	15.00	15.00
Chicken Fat with Dadex[1]	9.72	11.51	12.15	11.69
Pea Fibre	5.99	5.85	5.35	5.77
Pea Protein 48% (Prestige)	-	9.30	15.00	10.38
Potassium Chloride	0.78	0.98	1.18	1.00
Celite	1.00	1.00	1.00	1.00
Sodium Chloride	0.10	0.50	0.50	0.50
Calcium carbonate	-	0.38	0.46	0.41
Choline chloride	0.10	0.20	0.30	0.30
Taurine	0.10	0.10	0.10	0.10
Methionine D/L	0.10	0.11	0.20	0.13
Mineral Premix (Dog/Cat)[2]	0.10	0.10	0.10	0.10
Vitamin Premix (Cat)[2]	0.10	0.10	0.10	0.10
Dicalcium Phosphate	-	0.92	1.37	1.08

[1]Dadex = an antioxidant solution for animal fats to help extend shelf life of ingredient and pet food.
[2]Wheat, magnesium oxide, zinc, methionine, vitamin C, Alltech Bio-Mos, vitamin E, zinc sulphate, ferrous sulphate, iron proteinate, vitamin D3, Alltech deodorase, mineral oil, copper proteinate, copper sulphate, niacin, selenium enriched yeast, calcium iodate, vitamin A, manganese proteinate, calcium pantothenate, biotin, vitamin B12, riboflavin, manganese oxide, thiamine, sodium selenite, pyridoxine, folic acid.

The animals were fed the formulated diets for seven consecutive days, and fecal samples were collected on days eight and nine using a randomized latin-square cross-over design.

Proximate nutrient analyses of digestibility diets and fecal samples were performed by Central Testing Laboratories Inc (Winnipeg, MB, Canada). Results for proximate analyses of digestibility diets are shown in Table 4.3. The following was measured according to AOAC (Association of Official Analytical Chemists) and AOCS (American Oil Chemist Society) methods: ash (AOAC 923.03), crude protein (AOAC 990.03), fat (AOCS Am 5-04), crude fibre (AOCS Ba6a-05), acid insoluble ash to determine Celite levels (modified AOAC 920.08) and moisture (AOAC 930.15). Nitrogen free extract (NFE) was calculated using the following formula: % NFE = % DM – (% protein + % fat + % crude fibre + % ash). ME (dry matter) was calculated as ME = DE x (1.003 – (0.0021 x %CP)), where DE = 4151 – (122 x %Ash) + (23 x %CP) + (38 x %EE) – (64 x %CF) (National Research Council. 1998). The total tract apparent digestibility coefficient (TTADC) of the starches were calculated using the following formula as a percentage of dry matter:

Equation 4.1:

$$Nutrient\ digestibility\ (\%) = \left[1 - \left[\frac{\%\ nutrient\ in\ feces\ \times \%\ Celite\ in\ food}{\%\ nutrient\ in\ food\ \times \%\ Celite\ in\ feces}\right]\right] \times \%\ nutrient\ in\ food$$

4.2.2. Long-term Feeding Diets

During digestibility testing, it was observed that the cats did not like to eat or in a few cases, refused to eat the test diets, whereas dogs readily ate all diets. Because of this difficulty with the cats, the sample size for the digestibility trial is lower than this subsequent long-term

feeding trial and a comparable smaller number used in dogs. In order to improve palatability, test diets were re-formulated with ingredients that had higher palatability (more fish meal and fish oil, less soybean meal; see Table 4.2 for formulation and Table 4.4 for proximate analyses). Dry and wet palatants (AFB International, St.Charles, MO, USA) were also added to the long-term feeding diet formulations to enhance palatability for the cats (Dry palatant: Dry cat palatability enhancer BioFlavor F24047, Wet palatant: Liquid Cat palatability enhancer, Optimizor LC 647). Four complete test diets were formulated to be used in both species containing either modified corn, pea, faba bean or lentil starch at 30% inclusion for the long-term feeding study. The animals were randomized in a latin-square design, then fed one of the diets for six consecutive weeks. Portions were determined based on historic energy intake. Initial portions of the four test diets were equal in energy intake with the standard commercial diet (Hill's Science Diet, Hill Pet Nutrition, Inc. Topeka, USA) that was used prior to the study and during the washout period to maintain a healthy weight. The commercial diet used for basic husbandry when animals were not on trials had a reported caloric content of 3659 kcal/kg for the dog diet, and 4011 kcal/kg for the cat diet. The animals were weighed on a weekly basis and if needed, portion sizes were adjusted by 10% to either increase or decrease energy intake to maintain an ideal bodyweight. Calculations were done for average daily food intake (g/day; Equation 4.2) and average daily caloric consumption (kcal/day; Equation 4.3). At the end of the six weeks, blood was collected in animals fasted overnight for small animal blood chemistry, complete blood cell count and differential, plasma methylglyoxal (toxic glucose metabolite) and plasma nitrotyrosine (indicator of oxidative stress).

Table 4.2 Diet formulations used for long-term feeding trial in both cats and dogs. Test diets were nutritionally complete with 30% inclusion of modified corn, pea, faba bean or lentil starch.

Ingredient	Corn Diet (%)	Pea Diet (%)	Faba bean Diet (%)	Lentil Diet (%)
Cornstarch	30.00	-	-	-
Pea Starch	-	30.00	-	-
Faba bean Starch	-	-	30.00	-
Lentil Starch	-	-	-	30.00
Chicken meal	35.78	25.46	23.76	24.95
Soy Protein concentrate	9.31	15.00	15.00	15.00
Chicken Fat with Dadex[1]	7.75	9.76	10.44	9.84
Pea Fibre	4.96	6.88	6.89	6.90
Fish meal, mixed	5.00	5.00	5.00	5.00
AFB LC647[2]	2.00	2.00	2.00	2.00
Fish Oil	2.00	2.00	2.00	2.00
Potassium Chloride	0.90	0.88	1.00	1.00
Celite	1.00	1.00	1.00	1.00
Sodium Chloride	0.30	0.50	0.50	0.50
AFB F24047 Dry[2]	0.50	0.50	0.50	0.50
Calcium carbonate	0.00	0.41	0.55	0.43
Choline chloride	0.10	0.20	0.43	0.39
Taurine	0.10	0.10	0.10	0.10
Methionine D/L	0.10	0.10	0.10	0.10
Mineral Premix (Dog/Cat)[3]	0.10	0.10	0.10	0.10
Vitamin Premix (Cat)[3]	0.10	0.10	0.10	0.10
Dicalcium Phosphate	-	0.01	0.53	0.09

[1]Dadex = an antioxidant solution for animal fats to help extend shelf life of ingredient and pet food
[2]Dry palatant: Dry cat palatability enhancer BioFlavor F24047, Wet palatant: Liquid Cat palatability enhancer, Optimizor LC 647. Added to improve palatability of diets.
[3]Wheat, magnesium oxide, zinc, methionine, vitamin C, Alltech Bio-Mos, vitamin E, zinc sulphate, ferrous sulphate, iron proteinate, vitamin D3, Alltech deodorase, mineral oil, copper proteinate, copper sulphate, niacin, selenium enriched yeast, calcium iodate, vitamin A, manganese proteinate, calcium pantothenate, biotin, vitamin B12, riboflavin, manganese oxide, thiamine, sodium selenite, pyridoxine, folic acid.

Table 4.3 Proximate analyses of diets used for digestibility testing in both cats and dogs. Test diets were nutritionally complete with 30% inclusion of modified corn, pea, faba bean or lentil starch.

Nutrient	Corn Diet	Pea Diet	Faba bean Diet	Lentil Diet
DM (%)	95.68	97.06	96.62	97.88
Starch (%)	15.33	20.07	18.50	20.79
NFE (%)	34.42	32.65	31.39	33.91
Crude Protein (%)	37.67	37.54	36.44	35.38
Crude Fibre (%)	3.00	3.17	4.02	3.25
Fat (%)	15.28	17.09	18.58	17.75
Ash (%)	8.69	8.58	8.62	8.75
Acid Insoluble Ash (%)	1.13	1.18	1.23	1.16
ME(kcal/kg)	4982.82	5077.23	5137.24	5076.54
DM, dry matter; ME, metabolizable energy. Values shown as a dry matter basis.				

Table 4.4 Proximate analyses of diets used for long-term feeding trial in both cats and dogs. Test diets were nutritionally complete with 30% inclusion of modified corn, pea, faba bean or lentil starch.

Nutrient	Corn Diet	Pea Diet	Faba bean Diet	Lentil Diet
DM (%)	97.51	97.20	97.41	96.71
Starch (%)	19.06	21.43	19.33	23.28
NFE (%)	37.45	33.96	33.04	33.52
Crude Protein (%)	36.15	36.76	37.28	37.68
Crude Fibre (%)	2.68	3.31	3.31	3.22
Fat (%)	12.4	14.83	14.88	14.15
Ash (%)	10.35	10.17	10.51	10.45
Acid Insoluble Ash (%)	1.27	1.31	1.23	1.05
ME(kcal/kg)	3726.59	3802.43	3773.15	3763.71
DM, dry matter; ME, metabolizable energy. Values shown as a dry matter basis.				

An OGTT (15% w/v solution; 1 g/kg dose) was then performed, followed by a glycemic response to a single feeding challenge of the test diet (1 g/kg available carbohydrate in the test meal) at least three days later. Available carbohydrate was determined using a commercially available kit (Megazyme International, Ireland; Chapter 3). Cardiovascular responses (blood pressure, heart rate) were measured at times 0 and 60 minutes during these glycemic tests. This was followed by a two-week wash out period in which the animals consumed the standard commercial diet. The process was then repeated in a crossover design until all of the four test diets were consumed by all animals (n=8 beagles, n=9 cats). All diets were randomized and the individual performing the testing and data analysis was blinded to the treatment order.

Equation 4.2:

$$Average\ daily\ food\ intake = \frac{Animal1 + Animal2 + Animal3 + Animal4 + \cdots}{number\ of\ animals}$$

(Animal1 = g day 1 + g day 2 + g day 3…/ days)

Equation 4.3:

$$Average\ daily\ caloric\ consumption = (\frac{kcal}{g}) \times (\frac{g}{day})$$

4.2.3. Blood Collection and Plasma Analysis

Animals were fasted overnight, and aseptically catheterized using an intravenous catheter inserted into the cephalic vein. Blood samples were collected (2 ml for dogs; 1 ml for cats) and placed into tubes containing K^2 EDTA and centrifuged for five minutes at 5600 rpm (Statspin

Express 3, Beckman Coultier Inc., USA) to separate plasma for analysis. Emla cream (2.5% Lidocaine) was used on the cats only as a topical anaesthetic to help with ease of catheterization. No other anaesthetics or sedatives were used in this study. Blood was collected for the dogs at 0 (pre-feeding) and 15, 30, 45, 60, 90, 120, 150, 180 minutes postprandial (Adolphe et al. 2012); and for the cats at 0 (pre-feeding) and 15, 30, 60, 120, 180, 240, 300 minutes postprandial, determined from preliminary OGTT. All samples were analyzed for glucose and insulin. Plasma for methylglyoxal was collected at pre-feeding (fasted) versus 60 minutes (peak blood glucose time), and nitrotyrosine analyzed in pre-feeding (fasting) samples only. Following the six weeks of feeding test diets, an OGTT was performed followed by a single feeding test of the corresponding diet within the next three days. Following collection, catheters were flushed with a 1 ml 3% sodium citrate (Omnipure, Darmstadt, Germany), 0.9% NaCl (Sigma Aldrich, St.Louis, USA) sterilized solution for patency of catheter and fluid replacement.

4.2.3.1.Glucose assay

Plasma glucose analysis was performed using a glucose oxidase assay method and reagents from Sigma Aldrich (St.Louis, MO, USA) and analyzed on a Spectra Max 190 Microplate reader (Molecular Devices, LLC. Sunnyvale, CA, USA). Plasma glucose was determined at all time points for all treatments. To measure the GI of each of the whole formulated diets, incremental AUC was calculated using the trapezoid rule and expressed as a percent of the AUC after glucose challenge (Wolever et al. 1991). Glucose AUC was also calculated from the OGTT done after the six weeks of feeding. To calculate the GI for the chronic study, the baseline postprandial plasma glucose AUC calculated from the acute study was used because this was measured prior to the long-term study and has no associated effects from the long-term feedings.

4.2.3.2.Insulin assay

Plasma insulin analysis was performed using enzyme linked immunosorbent assays (ELISA) purchased from Mercodia Inc. (Uppsala, Sweden). A canine specific ELISA was used for the canine plasma samples, while a human insulin ELISA, validated for use in domestic felines, was used for the feline samples. Results were analyzed on a Spectra Max 190 Microplate reader. Plasma insulin was determined at all times points for all treatments. Again, the incremental AUC was calculated for each treatment using the trapezoid rule (Wolever et al. 1991).

4.2.3.3.Methylglyoxal Measurement

Plasma methylglyoxal was analyzed using high performance liquid chromatography (HPLC) methods previously described (Wang et al. 2005) and previously validated in canine serum samples (Adolphe et al. 2012). Samples were analyzed in duplicate, with pre-feeding (time 0) and 60 minutes postprandial plasma MG measured during OGTT and diet glycemic response testing. Results are expressed as a change in plasma MG between pre-feeding and 60 min postprandial levels.

4.2.3.4.Nitrotyrosine

Nitrotyrosine levels were assessed as a measure of oxidative stress, using an ELISA kit from Abcam (Cambridge, UK). Plasma was used from time 0 (pre-feeding), prior to the OGTT following the six-week feeding trial of each of the diets in all of the animals.

4.2.4. Blood Pressure and Heart Rate

Blood pressure and heart rate was determined by high definition oscillometry using a Vet Memodiagnostic HDO monitor (S + B medVET, Germany) and analyzed using MDS Win Analyse software version 2.1.2.1. The cuff was placed at the base of the tail, and measurements were taken at time 0 (pre-feeding) and 60 minutes postprandial following each treatment in all animals. An average of three measurements were used for analysis of systolic and diastolic pressures and heart rate according to methods previously used in dogs (Adolphe et al. 2012).

4.2.5. Statistical Analysis

Data (means ± SEM) were analyzed by IBM SPSS version 20.0 (International Business Machine Corp., USA). Related samples t-test was used to determine differences for cardiovascular measurements. One-way repeated measures ANOVA was used to determine differences among treatments for blood chemistry panel and complete blood count, glucose, insulin, MG and nitrotyrosine. Mauchly's test was used to test for sphericity. Post hoc analysis was performed using Fisher's least significant difference (LSD) to determine any pairwise differences. A $p<0.05$ was considered significant. Independent samples t-test were used to determine species differences within a treatment for the glucose, insulin, MG and nitrotyrosine. Levene's test was used to determine equal variances.

4.3. Results

4.3.1. Proximate Analyses of Long-term Feeding Diets

The goal of formulation of diets for long-term feeding trials in the cats and dogs was to keep energy density, protein, fat and starch content as similar as possible, but to vary the major starch source at 30% inclusion. Proximate analyses (Table 4.4) showed that the diets were isoenergetic with less than 80 kcal/kg difference between diets. They were also isonitrogenous

with less than 1.4% difference in crude protein between the diets. Starch content varied less than 5% between diets.

4.3.2. Total Tract Apparent Digestibility

Comparing the two diet formulations used in this study (Table 4.3 & 4.4) proximate analysis shows that both are within 4% of each other for NFE, crude fat and crude protein. Metabolizable energy (ME), calculated from proximate analysis data (National Research Council. 1998) for the digestibility diets (Table 4.3) was higher than the ME for the long-term feeding diets (Table 4.4). This may be due to the increases in fat for the digestibility diets since fat is known to provide 9 kcal/g of energy compared to 4 kcal/g for carbohydrates and protein (FAO. 2003). The total tract apparent digestibility coefficient (TTADC) of the digestible energy (DE), protein, fat and starches are shown in Table 4.5. Comparing among the diets, the digestibility for the starch in the modified cornstarch diet was significantly lower than all other starches in the diets for dogs (pea $p=0.007$; faba bean $p=0.008$; lentil $p=0.034$). For starch digestibility in cats, the modified cornstarch and faba bean diets were not significantly different ($p=0.079$). The digestibility of starch in the pea and lentil diets were significantly higher in cats than the modified cornstarch and faba bean diets. Also in cats, we see the energy digestibility of the modified cornstarch diet was significantly lower ($p=0.001$ for all) than all other diets. The digestibility of the starches in cats were similar or surprisingly tended to be higher than that in dogs, except for faba bean diet. When comparing starch digestibility between species the only diet that had a significant difference was the modified cornstarch diet, where the dogs had a significantly lower digestibility than the cats ($p = 0.013$). Energy digestibility was not different between diets for dogs. The digestibility of protein and fat was not significantly different between any of the diets in either species.

Table 4.5 Total tract apparent digestibility of whole formulated diets in dogs and cats.

DOGS				
Diet	DE (kcal/kg)	Protein (% DM)	Fat (% DM)	Starch (% DM)
Corn	4280.4 ± 43.5	84.9 ± 2.4	98.5 ± 0.7	68.0 ± 1.9[a]
Pea	4507.0 ± 26.7	84.6 ± 2.4	98.9 ± 0.4	80.6 ± 2.9[b]
Faba bean	4462.6 ± 27.7	83.8 ± 1.0	98.0 ± 0.6	83.2 ± 1.4[b]
Lentil	4456.1 ± 96.6	84.1 ± 1.2	97.9 ± 0.3	83.4 ± 3.4[b]
CATS				
Diet	DE (kcal/kg)	Protein (% DM)	Fat (% DM)	Starch (% DM)
Corn	4363.2 ± 8.4[a]	87.5 ± 0.4	96.8 ± 0.9	75.7 ± 1.0[a]
Pea	4614.3 ± 24.7[b]	89.0 ± 0.8	98.3 ± 0.2	85.8 ± 0.4[b]
Faba bean	4588.1 ± 32.0[b]	87.8 ± 0.4	97.6 ± 0.6	79.3 ± 1.4[a]
Lentil	4592.4 ± 24.1[b]	87.6 ± 0.8	98.2 ± 0.2	86.7 ± 0.4[b]

Protein, fat and starch reported on a dry matter basis. Results are shown as a mean ± SEM. Digestibility dogs n = 4; cats n = 5. DE, digestible energy; DM, dry matter. Values in a column with superscripts without a common letter differ; $p < 0.05$, one-way repeated measures ANOVA with LSD post hoc test.

No significant differences in digestibility for energy, protein and fat were observed between dogs and cats. Along with the reduced digestibility, the modified cornstarch diet reduced fecal quality in both dogs and cats (less solid with higher moisture content *i.e.* diarrhea).

4.3.3. Average Food Intake, Daily Energy and Animal Weight Change

Average daily food intake, average daily calorie intake, and weight change from before and after the six-week feeding trial for each diet are shown in Table 4.6 for both cats and dogs. Body weight (kg) was measured at the beginning of each feeding period and at the end of each six-week period to evaluate any significant changes due to the diet consumption. Dog results showed that only the lentil diet produced a significant weight loss from start to end after the six-week trial ($p=0.05$). For cats, the modified cornstarch diet ($p=0.044$) and the faba bean diet ($p=0.027$) both produced significantly lower body weights after six weeks. Despite changes, all cats and dogs started and ended each portion of the feeding trial within their ideal body condition (Dog BCS range 4-6, Cat BCS range 4-6; data not shown). One cat had to be removed from the modified cornstarch diet due to vomiting, diarrhea, limited food intake for two days, and eventual food refusal. Due to this, the modified cornstarch diet had an $n=8$, while the other diets had $n=9$.

Table 4.6 Dog and cat average daily food intake (g/day), average daily calorie consumption (kcal/day) and change in body weight (kg) from the beginning to the end of each six-week feeding trial in a cross-over study design following consumption of complete diets (modified corn, pea, faba bean and lentil starches).

	Corn Diet	**Pea Diet**	**Faba bean Diet**	**Lentil Diet**
DOG				
Weight change from start (kg)	-0.48 ± 0.24	-0.18 ± 0.13	-0.31 ± 0.18	-0.34 ± 0.14 *
Daily food intake (g/day)	182 ± 12	188 ± 9	175 ± 8	181 ± 16
Daily caloric consumption (kcal/day)	679	714	660	682
CAT				
Weight change from start (kg)	-0.14 ± 0.06 *	-0.02 ± 0.03	-0.07 ± 0.03*	+0.01 ± 0.04
Daily food intake (g/day)	47 ± 7	52 ± 3	45 ± 3	50 ± 4
Daily caloric consumption (kcal/day)	177	196	170	189

Dog (n=8); Cat (n=9 for pea, faba bean and lentil diets) (n=8 for modified cornstarch diet). Values shown are means ± SEM. Related samples t-test was performed to determine significant differences between before and after weights per diet; * denotes significance change in weight by end of feeding period for that diet ($p<0.05$).

4.3.4. Plasma Analysis

Blood chemistry panel and complete blood count for both dogs and cats taken at the end of each feeding period for each test diet are shown in Tables 4.7 and 4.8, respectively. These tables include reference values for each parameter and pre-testing values taken before the start of the long-term trial in both species. Baseline results are indicative of general health while maintained on the commercial diet (Science Diet, Hills Science). Looking at dog results first (Table 4.7), for the majority of ions, the four diets produced either significantly lower or similar results to the baseline, except for bicarbonate in which all four diets had significantly higher results. Despite this all ion values were in reference range. Urea was significantly lower from baseline for all four diets, but creatinine showed no differences. Pancreatic enzyme lipase showed no differences while amylase was significantly lower in all diets, except lentil, compared to baseline, but still well within reference range. Interestingly, fasting glucose was significantly higher after modified cornstarch, pea starch and lentil starch diets compared to baseline, and cholesterol was significantly lower for all diets compared to baseline, but again all within reference range. Liver enzymes are varied in responses, but all are within references ranges and not indicative of any liver changes. Total plasma protein was significantly lower than baseline after the pea starch and lentil starch diets, while albumin was significantly higher for the baseline compared to all four diets. Conversely, for plasma globulin, pea starch diet was significantly lower than faba bean starch diet, but all others showed no significant differences. Red blood cell count was significantly lower following all four diets compared to baseline.

Cat blood chemistry results for ions varied when comparing the baseline results to each of the diets (Table 4.8).

Table 4.7 Dog blood chemistry panel and complete blood count following six weeks of feeding of one of the four complete diets (modified corn, pea, faba bean and lentil starches) in a crossover study design.

		Diet				
	Reference Range	Baseline	Corn	Pea	Faba	Lentil
Blood Parameters						
Sodium (mmo/L)	140-153	150^{a}	148^{b}	146^{b}	147^{b}	148^{b}
Potassium (mmol/L)	3.8-5.6	5.0^{a}	4.5^{b}	4.6^{b}	4.6^{b}	4.6^{b}
Na:K Ratio	28-38	30^{a}	32^{ab}	32^{b}	32^{b}	32^{b}
Chloride (mmol/L)	105-120	113^{a}	112^{ab}	110^{b}	111^{ab}	111^{ab}
Bicarbonate (mmol/L)	15-25	19^{a}	21^{b}	21^{b}	21^{b}	21^{b}
Anion Gap (mmol/L)	12-26	23^{a}	20^{b}	20^{b}	20^{b}	20^{b}
Calcium (mmol/L)	1.91-3.03	2.60^{a}	2.59^{ab}	2.51^{b}	2.53^{b}	2.54^{ab}
Phosphorus (mmol/L)	0.63-2.41	1.68^{a}	1.42^{ab}	1.30^{b}	1.33^{b}	1.27^{b}
Magnesium (mmol/L)	0.7-1.16	0.88^{a}	0.82^{b}	0.83^{b}	0.84^{b}	0.84^{b}
Urea (mmol/L)	3.5-11.4	8.2^{a}	7.0^{b}	6.5^{bc}	6.5^{c}	6.6^{bc}
Creatinine (µmol/L)	41-121	62	62	63	61	61
Amylase (U/L)	343-1375	588^{a}	511^{b}	500^{b}	527^{b}	513^{ab}
Lipase (U/L)	25-353	40	40	64	63	45
Glucose (mmol/L)	3.1-6.3	3.7^{a}	4.6^{b}	4.5^{b}	4.4^{ab}	4.4^{b}
Cholesterol (mmol/L)	2.7-5.94	4.95^{a}	3.92^{b}	3.81^{bc}	4.04^{b}	3.67^{c}
Total Bilirubin (µmol/L)	1.0-4.0	1.1^{a}	1.2^{ab}	1.3^{ab}	1.6^{b}	1.4^{ab}
Direct Bilirubin (µmol/L)	0.0-2.0	0.5	0.4	0.3	0.4	0.4
Indirect Bilirubin (µmol/L)	0.0-2.5	0.6^{a}	0.7^{ab}	1.0^{ab}	1.2^{b}	1.0^{b}
Alk Phos (U/L)	9-90	69^{a}	22^{bd}	26^{bc}	29^{c}	22^{d}
GGT (U/L)	0-8	1^{a}	7^{ab}	3^{ab}	2^{b}	5^{ab}
ALT (U/L)	19-59	28^{ab}	32^{b}	25^{ac}	24^{c}	25^{ac}
GLDH (U/L)	0-7	5^{a}	6^{ab}	3^{ab}	4^{ab}	3^{b}
CK (U/L)	51-418	168	162	169	191	161
Total Protein (g/L)	55-71	55^{a}	54^{abc}	53^{bc}	54^{ad}	54^{cd}
Albumin (g/L)	32-42	35^{a}	34^{b}	34^{b}	34^{b}	34^{b}
Globulin (g/L)	20-34	20^{ab}	20^{ab}	19^{a}	20^{b}	20^{ab}
A:G Ratio	1.06-1.82	1.83^{a}	1.72^{ab}	1.83^{ab}	1.70^{b}	1.74^{ab}
WBC (x 10^{9}/L)	4.9-15.4	6.0	6.0	5.9	5.1	5.9
RBC (x 10^{12}/L)	5.8-8.5	7.12^{a}	6.00^{b}	5.98^{b}	5.98^{b}	6.09^{b}

Dogs (n=8); Values are means from blood collected at the end of six weeks of feeding one of the four diets. Values in a row with superscripts without a common letter differ. No letters indicates there are no significant differences; $p < 0.05$, one-way repeated measures ANOVA with LSD post hoc test.

Table 4.8 Cat blood chemistry panel and complete blood count following six weeks of feeding of one of the four complete diets (modified corn, pea, faba bean, lentil) in a crossover study design.

		Diet				
	Reference Range	Baseline	Corn	Pea	Faba	Lentil
Blood Parameters						
Sodium (mmol/L)	147-160	154^{a}	152^{b}	152^{b}	152^{b}	153^{ab}
Potassium (mmol/L)	3.9-5.5	4.0^{a}	4.8^{b}	5.1^{c}	5.1^{c}	5.0^{bc}
Chloride (mmol/L)	111-125	117^{a}	116^{b}	116^{b}	115^{b}	115^{ab}
Bicarbonate (mmol/L)	11-22	14^{a}	18^{bc}	17^{b}	18^{bc}	19^{c}
Anion Gap (mmol/L)	15-30	26^{a}	23^{ab}	25^{b}	24^{ab}	24^{ab}
Calcium (mmol/L)	2.26-2.86	2.58^{ab}	3.02^{c}	2.56^{a}	2.70^{abc}	2.69^{b}
Phosphorus (mmol/L)	1.08-2.21	1.37^{a}	1.57^{b}	1.68^{b}	1.65^{b}	1.66^{b}
Magnesium (mmol/L)	0.74-1.12	0.90^{a}	1.02^{b}	0.95^{bc}	0.98^{bc}	0.94^{ac}
Urea (mmol/L)	6.0-11.4	7.6^{a}	9.2^{b}	8.3^{ab}	8.4^{b}	8.4^{b}
Creatinine (µmol/L)	78-178	136^{ab}	158^{c}	130^{a}	141^{b}	131^{a}
Glucose (mmol/L)	3.5-8.1	4.3	4.3	4.1	4.3	4.5
Cholesterol (mmol/L)	1.62-4.32	5.12^{a}	3.95^{c}	4.32^{ac}	4.48^{ab}	4.28^{bc}
Total Bilirubin (µmol/L)	0.0-3.0	1.1^{a}	0.7^{b}	0.4^{b}	0.8^{b}	0.6^{b}
Alk Phos (U/L)	11-56	20^{a}	17^{ab}	18^{a}	14^{b}	14^{b}
GGT (U/L)	0-6	0	0	0	0	0
ALT (U/L)	22-90	65^{a}	67^{a}	49^{b}	40^{b}	47^{b}
GLDH (U/L)	1-5	2	3	2	2	2
CK (U/L)	75-471	870^{ab}	146^{ac}	171^{ad}	181^{bd}	200^{bc}
Total Protein (g/L)	53-84	72	73	71	72	71
Albumin (g/L)	28-43	36	37	36	36	36
Globulin (g/L)	23-45	36	36	36	36	35
A:G Ratio	0.77-1.64	1.03	1.02	1.02	1.02	1.05
WBC (x 10^{9}/L)	4.2-13.0	13.5	12.6	12.0	11.5	11.9
RBC (x 10^{12}/L)	6.2-10.6	9.56^{a}	8.53^{ab}	9.06^{ab}	8.17^{b}	8.81^{ab}

Cats (n=9 for pea, lentil and faba bean diets; n=8 for modified cornstarch diet); Values are means from blood collected at the end of six weeks of feeding one of the four diets. Values in a row with superscripts without a common letter differ. No letters indicates there are no significant differences; $p < 0.05$, one-way repeated measures ANOVA with LSD post hoc test.

All ion values were within reference range for cats except for calcium which was significantly higher following the modified cornstarch diet than pea starch, lentil starch or baseline diets. Urea baseline was significantly lower in cats than all diets except pea starch diet. On the other hand, creatinine was significantly higher following modified cornstarch diet compared to all other diets and baseline. Cholesterol was highest for baseline, but only significantly different from modified cornstarch and lentil starch diets in cats. Liver enzymes trends were lower or unchanged following consumption of the pulse starch diets compared to baseline or modified cornstarch diet in cats. An exception was creatine kinase that was out of range high for baseline results, but within range and with no significant differences among test diets in cats. The high creatine kinase values at baseline likely came from two stressed cats at that time showing levels >2500 U/L, with normal levels in all subsequent tests. No differences were seen in blood protein levels between baseline and the four diets. Red blood cell results are highest in the baseline in cats, but only significantly different from the faba bean diet.

The blood chemistry panel results showed a few parameters that are out of reference range following the six-week crossover study of the test diets. In dogs total protein was below reference range for all of the diets, and A:G ratio was higher than the reference range for the pea starch diet. In cats, calcium was higher than the clinical reference range following feeding of the modified cornstarch diet, and cholesterol was higher than reference range following feeding of the faba bean starch diet. All other haematological parameters were within reference range for all of the diets in both species.

Plasma glucose results from the GTT and following the single feeding challenge of test diet for dogs and cats are shown in Tables 4.9 and 4.10, and the time course of glycemic responses are shown in Figures 4.1 (dog) and 4.2 (cat). Fasting blood glucose levels for dogs were: lentil starch 4.19 ± 0.03, modified cornstarch 4.24 ± 0.02, faba bean starch 4.38 ± 0.05, and pea starch 4.43 ± 0.05. After six weeks, fasting blood glucose for the lentil starch diet was significantly lower than faba bean starch and pea starch diets, where as modified cornstarch diet was only significantly different than pea starch diet. Cat fasting blood glucose levels are: pea starch 4.48 ± 0.06, modified cornstarch 4.59 ± 0.24, lentil starch 4.65 ± 0.26 and faba bean starch 4.67 ± 0.08. After six weeks on the diets there were no significant differences seen in fasting blood glucose levels. After six weeks on test diets, the OGTT showed no significant differences in peak blood glucose or glucose AUC among diets in either dogs or cats. In dogs, the time to peak blood glucose was significantly longer ($p = 0.048$) after being fed the pea starch diet compared to the faba bean starch diet, while modified cornstarch and lentil starch diets produced intermediate results. In contrast, there were no significant differences among the diets in cat blood glucose time to peak after GTT. Blood glucose results following the single feeding of the whole diets showed no significant differences for peak, AUC and GI in both dogs and cats among the diets. The only difference in GTT response was in dogs after being fed diets for six weeks, where blood glucose time to peak was significantly longer ($p = 0.041$) following the feeding of the modified cornstarch diet compared to the pea diet. No significant differences were seen in blood glucose time to peak in the cats among the diets.

Blood plasma insulin responses in dogs and cats following the GTT tested at the end of six weeks of feeding test diets are shown in Table 4.11 and results following the single feeding challenge of test diets are shown in Table 4.12.

Table 4.9 Postprandial glycemic responses in dogs and cats following an oral glucose tolerance test (15% w/v solution; 1 g/kg) after a six-week period of feeding the same test diet in a crossover study design.

	Corn diet	Lentil diet	Pea diet	Faba bean diet
DOGS				
Fasting glucose (mmol/L)	4.2 ± 0.02 ^{a,b}	4.2 ± 0.03 ^{a}	4.4 ± 0.1 ^{c}	4.4 ± 0.1 ^{b,c}
Peak (mmol/L)	6.6 ± 0.4	6.2 ± 0.4	6.8 ± 0.4	6.5 ± 0.3
Time to Peak (min)	32 ± 2 $^{a, b}$	30 ± 3 $^{a, b}$	36 ± 5 ^{a}	24 ± 3 ^{b}
AUC (mmol/L min)	128 ± 18	132 ± 23	116 ± 17	115 ± 18
CATS				
Fasting glucose (mmol/L)	4.6 ± 0.2	4.7 ± 0.3	4.5 ± 0.2	4.7 ± 0.1
Peak (mmol/L)	8.1 ± 0.7	7.3 ± 0.4	7.7 ± 0.5	7.6 ± 0.8
Time to Peak (min)	64 ± 11	53 ± 11	53 ± 5	54 ± 11
AUC (mmol/L min)	306 ± 72	231 ± 49	268 ± 52	272 ± 97

Results are shown in descending order of area under the curve (AUC) value for dogs; cat AUC values are aligned with the dog. Values are means ± SEM; n = 8 (dogs), n = 9 (cats) for pea, faba bean and lentil diets, n = 8 (cats) for modified cornstarch diet. Glucose was fed on a 1g per kg bodyweight basis. Values in a row with superscripts without a common letter differ; $p < 0.05$, one-way repeated measures ANOVA with LSD post hoc test.

Table 4.10 Postprandial glycemic responses in dogs and cats following single feedings of whole formulated diets (1 g/kg available carbohydrate) after a six-week period of feeding the same test diet in a crossover study design.

	Corn diet	Pea diet	Faba bean diet	Lentil diet
DOGS				
Fasting glucose (mmol/L)	4.2 ± 0.02 ^{a,b}	4.4 ± 0.1 ^{b,c}	4.4 ± 0.1 ^{c}	4.2 ± 0.03 ^{a}
Peak (mmol/L)	4.8 ± 0.1	4.6 ± 0.2	4.6 ± 0.2	4.6 ± 0.2
Time to Peak (min)	51 ± 4 ^{b}	38 ± 6 ^{a}	60 ± 12 $^{a, b}$	47 ± 7 $^{a, b}$
AUC (mmol/L min)	69 ± 19	44 ± 9	47 ± 13	44 ± 18
GI	58 ± 16	48 ± 16	40 ± 9	36 ± 16
CATS				
Fasting glucose (mmol/L)	4.6 ± 0.2	4.5 ± 0.2	4.7 ± 0.1	4.7 ± 0.3
Peak (mmol/L)	4.7 ± 0.2	4.4 ± 0.1	4.4 ± 0.2	4.6 ± 0.2
Time to Peak (min)	92 ± 29	120 ± 25	68 ± 12	62 ± 14
AUC (mmol/L min)	75 ± 23	40 ± 12	54 ± 19	67 ± 17
GI	44 ± 14	22 ± 5	28 ± 10	34 ± 10

Area under the curve (AUC); Results are shown in descending order of glycemic index (GI) value for dogs; cat glycemic index values are aligned with the dog. Values are means ± SEM; n = 8 (dogs), n = 9 (cats) for pea, faba bean and lentil starch diets, n = 8 (cats) for modified cornstarch diet. Diets were fed on a 1g available carbohydrate per kg bodyweight basis. Values in a row with superscripts without a common letter differ; $p < 0.05$, one-way repeated measures ANOVA with LSD post hoc test.

Table 4.11 Postprandial insulinemic responses in dogs and cats following an oral glucose tolerance test (15% w/v solution; 1 g/kg) after a six-week period of feeding the same test diet in a crossover study design.

	Corn diet	Lentil diet	Pea diet	Faba bean diet
DOGS				
Fasting insulin (pmol/L)	32 ± 3	29 ± 4	29 ± 5	33 ± 4
Peak (pmol/L)	222 ± 29[a]	170 ± 17 [b]	180 ± 16 [b]	174 ± 18 [a, b]
Time to Peak (min)	28 ± 3	30 ± 3	30 ± 3	23 ± 4
AUC (pmol/L min)	8530 ± 1624	8412 ± 1330	7922 ± 1014	6654 ± 776
CATS				
Fasting insulin (pmol/L)	41 ± 4[a]	23 ± 0.1[b]	28 ± 0.4[b]	21 ± 3[b]
Peak (pmol/L)	91 ± 6	88 ± 9	99 ± 15	79 ± 6
Time to Peak (min)	54 ± 18	24 ± 3	58 ± 15	36 ± 12
AUC (pmol/L min)	7115 ± 918	4874 ± 810	5964 ± 913	4978 ± 757

Results are shown in descending order of area under the curve (AUC) value for dogs; cat AUC values are aligned with the dog. Values are mean ± SEM; n = 8 (dogs), n = 9 (cats) for pea, faba bean and lentil starch diets, n = 8 (cats) for modified cornstarch diet. Glucose was fed on a 1g per kg bodyweight basis. Values in a row with superscripts without a common letter differ; $p < 0.05$, one-way repeated measures ANOVA with LSD post hoc test.

Table 4.12 Postprandial insulinemic responses in dogs and cats following a single feeding of whole diets (1g/kg available carbohydrate) after a six-week period of feeding the same test diet in a crossover study design.

	Corn diet	Lentil diet	Pea diet	Faba bean diet
DOGS				
Fasting insulin (pmol/L)	32 ± 3	29 ± 4	29 ± 5	33 ± 4
Peak (pmol/L)	115 ± 9	128 ± 22	114 ± 10	111 ± 10
Time to Peak (min)	54 ± 9	51 ± 5	51 ± 6	53 ± 4
AUC (pmol/L min)	8222 ± 1143	7628 ± 1383	7276 ± 977	6130 ± 1306
CATS				
Fasting insulin (pmol/L)	41 ± 4[a]	23 ± 0.1[b]	28 ± 0.4[b]	21 ± 3[b]
Peak (pmol/L)	83 ± 19 [a]	85 ± 15 [a, b]	55 ± 12 [b]	59 ± 12 [a, b]
Time to Peak (min)	75 ± 28	39 ± 13	64 ± 35	36 ± 7
AUC (pmol/L min)	5954 ± 832 [a]	9031 ± 1708[b]	6588 ± 2061[a,b]	5243 ± 1508 [a]

Results are shown in descending order of area under the curve (AUC) value for dogs; cat AUC values are aligned with the dog. Values are means ± SEMs; n = 8 (dogs), n = 9 (cats) for pea, faba bean and lentil diets, n = 8 (cats) for modified cornstarch diet. Diets were fed on a 1g available carbohydrate per kg bodyweight basis. Values in a row with superscripts without a common letter differ; $p < 0.05$, one-way repeated measures ANOVA with LSD post hoc test.

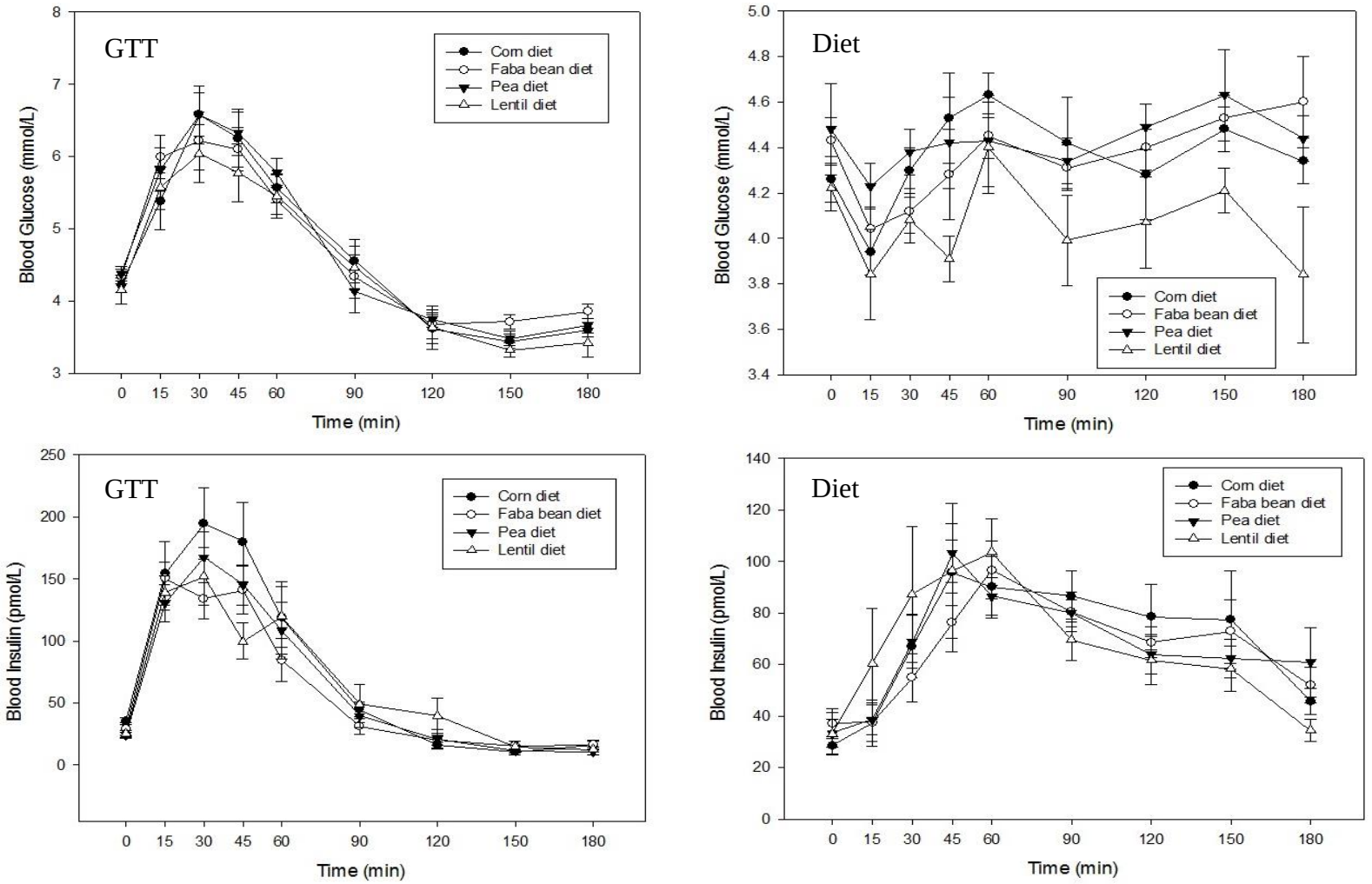


Figure 4.1 Dog (n=8) blood plasma glucose and insulin time course curves following single feedings of a glucose control (15% w/v solution; 1 g/kg) in an oral glucose tolerance test and single feedings of the whole diets after six weeks of long-term feeding. Dogs were fed 1 g available carbohydrate per kg BW of each diet. Values are means ± SEM.

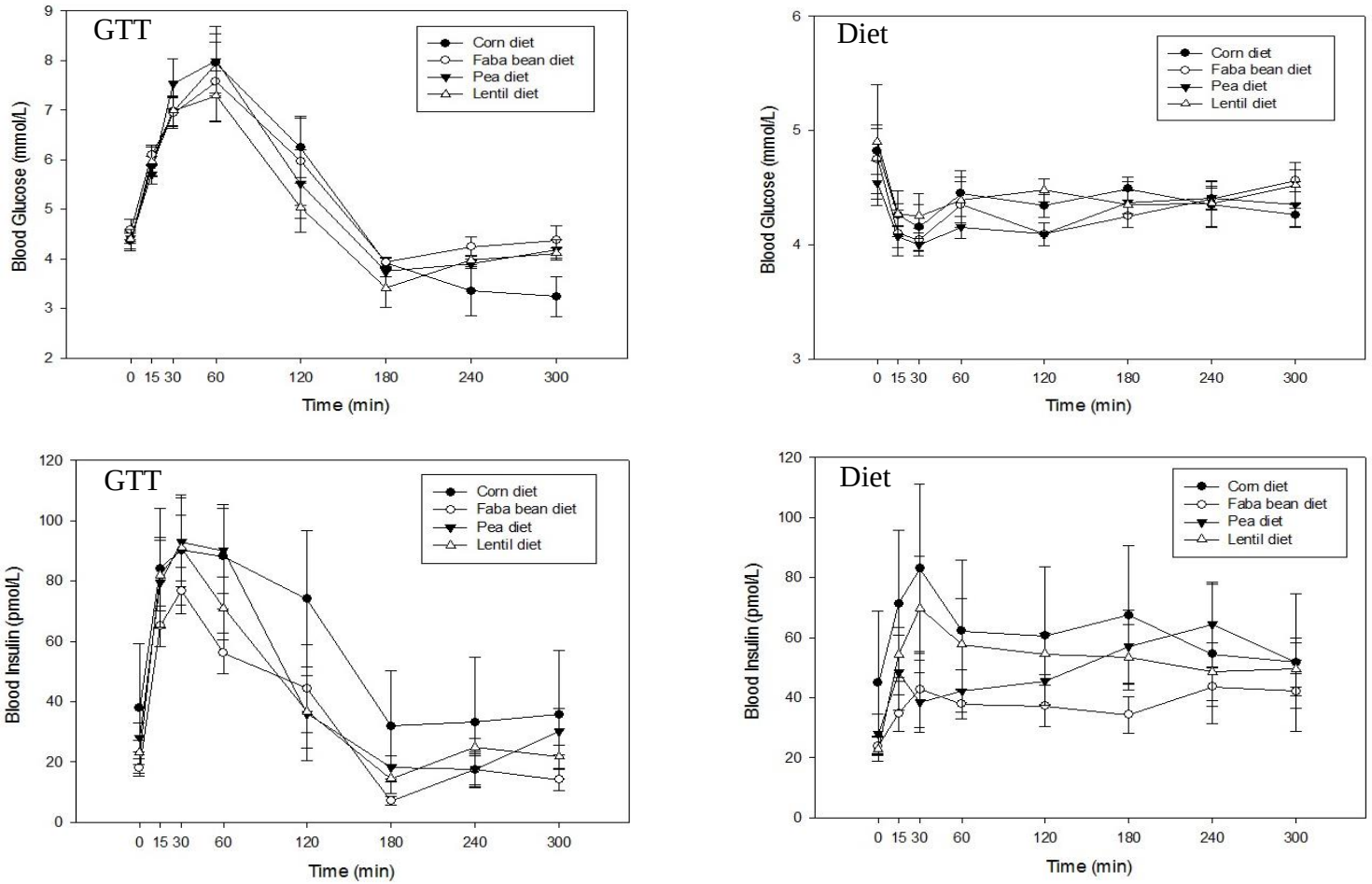


Figure 4.2 Cat (n = 9 for pea, faba bean and lentil diets, n = 8 for modified cornstarch diet) blood plasma glucose and insulin time course curves following single feedings of a glucose control in an oral glucose tolerance test (15% w/v solution) and single feedings of the whole diets after six weeks of long-term feeding. Cats were fed 1 g available carbohydrate per kg BW of each diet. Values are means ± SEMs.

The time course of insulin responses after both the GTT and single diet feeding are shown in Figures 4.1 for dogs and 4.2 for cats. Dog fasting insulin values were: faba bean starch diet 33.14 ±3.91, modified cornstarch diet 31.75 ± 3.31, lentil starch diet 29.22 ± 3.79 and pea starch diet 28.59 ± 4.72. No significant differences were seen between diets for fasting insulin values. Fasting blood insulin values for cats were: modified cornstarch diet 41.29 ± 3.55, pea starch diet 27.63 ± 0.35, lentil starch diet 22.92 ± 0.11 and faba bean starch diet 20.92 ± 2.95. Modified cornstarch diet fasting insulin was significantly higher than all other diets. Postprandial peak insulin after GTT in dogs fed the modified cornstarch diet for six weeks was significantly higher than that of dogs that were fed pea starch or lentil starch diets. In contrast, the peak insulin response to GTT in dogs fed faba bean starch was not significantly different from any other diet. Postprandial peak insulin GTT results in cats showed no significant differences among diets. For both species there were no significant differences after GTT among any of the diets for time to peak insulin, although $p = 0.052$ for peak insulin after GTT in cats fed lentil starch diet compared to either modified cornstarch or pea starch diets. For both dogs and cats, insulin AUC after GTT showed no significant differences among diets. Insulin responses following the single feeding challenge of the corresponding test diet showed no significant differences for peak values, time to peak, and AUC in dogs. Cat responses after the single feeding challenge with test diet showed that the peak insulin was significantly higher ($p = 0.038$) after six weeks of feeding the modified cornstarch diet compared to the pea starch diet. In contrast, after six weeks of feeding faba bean starch or lentil starch diets in cats, the peak insulin response after a single feeding of test diet was intermediate compared to these other diets. In cats fed diets for six weeks, the time to peak insulin response after a single feeding of test diet showed no significant differences among the diets. In contrast, in cats fed the lentil starch diet for six weeks, a significantly higher insulin

AUC in response to a single feeding of test diet compared to both modified cornstarch ($p = 0.044$) and faba bean starch ($p = 0.016$) diets was observed.

Overall fasting MG values for dogs were 0.756 ± 0.017 μM, and cat fasting MG values were 0.698 ± 0.035 μM, with no statistically significant species difference or differences in fasting values at the end of each six-week test diet period within a species. Postprandial changes in plasma MG results for both dogs and cats after feeding test diets for six weeks are shown in Figure 4.3. In dogs, no significant differences among any of the diets were observed in postprandial plasma changes in MG following either the GTT or the single feeding testing. In cats fed test diets for six weeks, however, the postprandial change in plasma MG level following GTT was significantly higher in cats fed the modified cornstarch diet compared to faba bean starch diet ($p=0.045$), while responses in cats fed the other two pulse starch diets were not significantly different. Moreover, this difference in postprandial MG response disappeared when cats were challenged with single feedings of test diet since no significant differences among the diets were found. When comparing the magnitude of % change in MG levels between species we see that dogs tend to have higher postprandial changes, where as in the cats a general trend to decrease postprandial levels was found, except for the lentil starch diet.

Fasting plasma nitrotyrosine concentrations in dogs and cats after six weeks of feeding test diets are shown in Table 4.13. No significant differences were observed among any test diets in both species. However, cat plasma nitrotyrosine levels were significantly higher (modified cornstarch $p=0.011$, pea starch $p=0.004$, faba bean starch $p=0.013$, lentil starch $p=0.011$; almost 10-fold higher) overall compared to those in dogs.

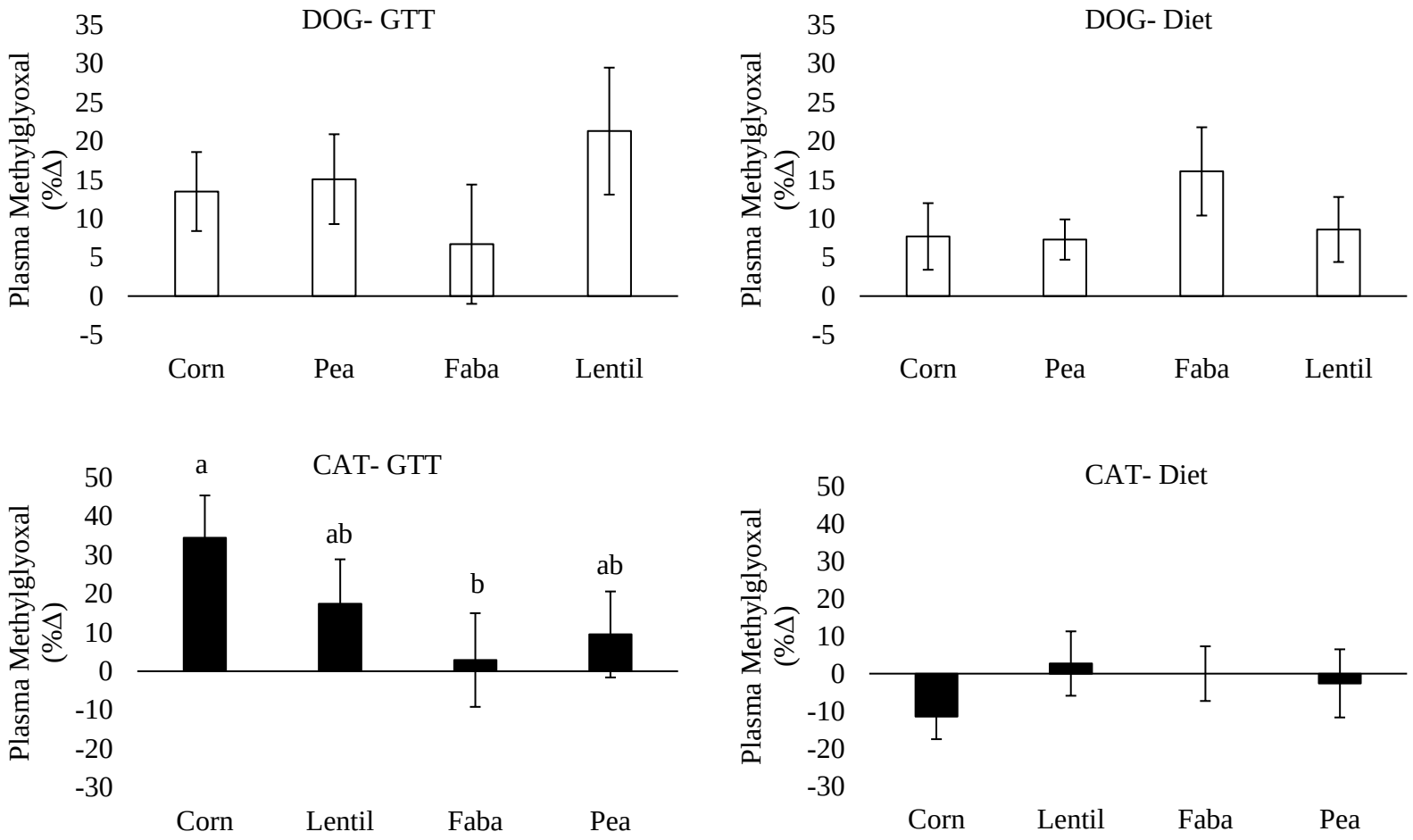


Figure 4.3 Plasma methylglyoxal levels as a percent change from time 0 (pre-feeding or fasting) to 60 minutes postprandial after a GTT and a single feeding of whole diets following the six-week feeding trial in a crossover study design. Dogs n=8, and Cats n=9 for pea, lentil and faba bean starch diets, n=8 for modified cornstarch diet. One-way repeated measures ANOVA followed by a LSD post hoc test was used to determine significant differences ($p<0.05$) among the groups. Groups without a common letter differ from each other. No letters indicates no significant differences seen among the diets. Values are means ± SEMs.

Table 4.13 Fasting plasma nitrotyrosine concentrations following six weeks of feeding of either modified corn, faba bean, pea or lentil starch diets with 30% starch inclusion in a crossover study design in both dogs and cats.

	Corn diet	Faba bean diet	Pea diet	Lentil diet
DOGS				
Average (nM)	4.43	4.34	3.78	4.27
SEM	0.25	0.67	0.60	0.48
CATS				
Average (nM)	31.91	32.01	30.36	35.82
SEM	9.04	8.72	6.72	9.56

N = 8 (dogs), n = 9 (cats) for pea, faba bean and lentil starch diets, n = 8 (cats) for modified cornstarch diet. No significant differences were seen between diets within a species.

4.3.5. Blood Pressure and Heart Rate

Blood pressure in dogs and cats after feeding test diets for six weeks are summarized in Table 4.14 (dogs) and Table 4.15 (cats). Values are reported for fasted animals and 60 minutes postprandial. When comparing fasting cardiovascular parameters between diets following six weeks of feeding the test diets in the dogs there were significant differences seen in diastolic pressure between pea starch diet and faba bean starch diet ($p=0.023$). There were no significant differences in baseline systolic pressure or heart rate among the diets in dogs. Comparing fasting cardiovascular parameters in cats following six weeks of feeding each of the diets, no significant differences were found between diets for systolic and diastolic pressures or heart rate. In dogs after feeding the pea starch diet for six weeks, a significant increase from fasting to 60 minutes postprandial was observed for both diastolic pressure and heart rate following the GTT. Similarly, significant postprandial increases in systolic and diastolic pressure were observed following the GTT in dogs fed the faba bean starch diet for six weeks. No other significant postprandial changes were observed in dogs fed the modified cornstarch or lentil starch diets. In contrast, in cats, significant postprandial increases in systolic pressure and heart rate following the GTT and a significant increase in heart rate after the single feeding of the faba bean starch diet was observed. Moreover, a significant postprandial increase in heart rate following the GTT and a significant postprandial increase in diastolic pressure were observed following the single diet feeding in cats after six weeks consumption of the pea starch diet. Interestingly, a significant postprandial decrease in systolic pressure following the single feeding of the lentil starch diet was observed in cats after six weeks.

Table 4.14 Cardiovascular parameters in dogs fed diets with 30% inclusion of modified corn, pea, lentil or faba bean starch for six weeks at times 0 (pre-feeding, fasting) and 60 minutes postprandial following either an oral glucose tolerance test or a single feeding of the whole diets.

Treatment	Time	Systole (mm Hg)	Diastole (mm Hg)	Pulse (bpm)
GTT				
Corn Diet	0	141 ± 5	65 ± 3[a,b]	66 ± 4
	60	141 ± 4	66 ± 2	74 ± 2
Pea Diet	0	137 ± 5	60 ± 3[a]	66 ± 4
	60	144 ± 5	64 ± 2[*]	74 ± 3[*]
Faba bean Diet	0	135 ± 6	64 ± 3[b]	75 ± 6
	60	146 ± 4[*]	69 ± 2[*]	78 ± 5
Lentil Diet	0	135 ± 6	61 ± 2[a,b]	69 ± 7
	60	137 ± 4	60 ± 3	75 ± 4
Diet Single feeding				
Corn Diet	0	139 ± 4	65 ± 2	66 ± 3
	60	143 ± 4	62 ± 3	72 ± 4
Pea Diet	0	138 ± 6	65 ± 3	71 ± 3
	60	129 ± 4	62 ± 4	68 ± 3
Faba bean Diet	0	132 ± 2	68 ± 3	71 ± 4
	60	137 ± 3	66 ± 3	74 ± 4
Lentil Diet	0	133 ± 4	63 ± 4	70 ± 5
	60	139 ± 5	64 ± 2	71 ± 4

Results are shown in decreasing glycemic index order. Values are mean ± SEM, n = 8. Time 60 values with a * indicate a significant difference from time 0 within a treatment; $p < 0.05$, a paired t-test was used to determine differences among related samples within a treatment. One-way repeated measures ANOVA was used to determine differences in baseline cardiovascular parameters; $p < 0.05$. Groups without a common letter differ from each other. No letters indicates no significant differences seen among the diets.

Table 4.15 Cardiovascular parameters in cats fed diets with 30% inclusion of modified corn, pea, lentil or faba bean starch for six weeks at times 0 (pre-feeding, fasting) and 60 minutes postprandial following either an oral glucose tolerance test or a single feeding of the whole diets.

Treatment	Time	Systole (mm Hg)	Diastole (mm Hg)	Pulse (bpm)
GTT				
Corn Diet	0	137 ± 5	65 ± 3	134 ± 13
	60	146 ± 4	72 ± 3	146 ± 13
Lentil Diet	0	146 ± 5	67 ± 3	139 ± 12
	60	143 ± 5	65 ± 3	147 ± 12
Faba bean Diet	0	139 ± 5	66 ± 2	140 ± 10
	60	154 ± 6*	68 ± 2	158 ± 13*
Pea Diet	0	143 ± 5	67 ± 2	137 ± 10
	60	148 ± 5	66 ± 3	151 ± 12*
Diet Single feeding				
Corn Diet	0	142 ± 4	67 ± 3	144 ± 11
	60	143 ± 6	70 ± 2	154 ± 8
Lentil Diet	0	141 ± 4	65 ± 2	146 ± 11
	60	135 ± 5*	65 ± 4	152 ± 8
Faba bean Diet	0	143 ± 5	67 ± 2	147 ± 10
	60	136 ± 4	65 ± 2	159 ± 11*
Pea Diet	0	138 ± 3	63 ± 2	143 ± 9
	60	144 ± 4	74 ± 4*	156 ± 14

Results are shown in decreasing glycemic index order. Values are means ± SEMs, n = 9. Time 60 values with a * indicate a significant difference from time 0 within a treatment; $p < 0.05$, a paired t-test was used to determine differences among related samples within a treatment. One-way ANOVA was used to determine differences in baseline cardiovascular parameters; $p < 0.05$. No letters indicates no significant differences seen among the diets.

4.4. Discussion

The major finding of this study was that after feeding the modified cornstarch diet for six weeks, dogs had decreased insulin sensitivity (higher peak insulin following a GTT), despite the diet having low digestibility and glycemic index, while diets containing pulse starches did not affect insulin sensitivity. In contrast, six weeks of feeding diets with both modified cornstarch and lentil starches in cats led to decreased insulin sensitivity (higher insulin peak for modified cornstarch diet, higher insulin AUC for lentil starch diet), suggesting that use of these starches in cat diets would not be as healthy long-term. Also, that pulse-based diets can promote weight control, in dogs, leading to a healthier lifestyle. Long-term feedings of test diets were done to evaluate health effects from pulse diets compared to a modified cornstarch-based diet over six weeks. There are numerous studies to date that have examined the health benefits of consuming a low GI diet in both healthy and diabetic humans, but less is known about the effects in dogs and cats. Pulses offer a healthy low GI option as an alternative to other high GI carbohydrates such as corn and rice. Despite ingredients such as peas and lentils increasingly being seen in pet foods due to the high interest in grain-free options, there is little to no long-term data on the health effects of these ingredients in pet diets. Thus, the major goal of this study was to evaluate whether diets utilizing pulse starches would offer a low GI healthy alternative carbohydrate option for both dogs and cats. Previous work in our group has demonstrated health benefits of utilizing whole pea flour in obese beagles (Adolphe et al. 2015), but to our knowledge, this is the first study that shows chronic health benefits of pulse-based diets in cats, as well as extending the findings in dogs to compare other pulses with our findings with peas.

4.4.1. Total Tract Apparent Digestibility

Digestibility of modified cornstarch and pulse starches were determined as a first step before using these same starch sources at 30% inclusion in complete diets for long-term feeding trials in dogs and cats. Modified (hydroyxpropyl-linked) cornstarch was used instead of unmodified cornstarch in order to better mimic the pulse starches in terms of digestibility. This was confirmed experimentally in this thesis since modified cornstarch showed significantly lower starch digestibility compared to the three pulse starches. Unmodified cornstarch is highly digestible with 97.5% reported in cats and >98% in dogs in previous studies (Murray et al. 1999; Carciofi et al. 2008; de-Oliveira et al. 2008; Bazolli et al. 2015). Of all covalent modifications examined in starch, hydroxypropylation (HP) has been shown to result in the lowest eGI (Chung et al. 2008b), which agrees with the low digestibility values observed in the present study. Similarly, an *in vivo* study in rats using HP modified potato starch showed significantly reduced starch digestibility compared to native starch (Björck et al. 1989). Another important observation in both the current digestibility and long-term feeding studies was reduced fecal quality in both cats and dogs in diets with modified cornstarch. In this case, lower digestibility of modified cornstarch may be related to increased gut motility and diarrhea. Modified starches, because of the decrease in digestibility and presumed effect to decrease glycemic responses, have been suggested for treatment of diseases such as diabetes mellitus in humans (Wolf et al. 1999). However, due to this effect to lower stool quality, the lower digestibility of pulse starches would be preferred in dogs and cats over use of a modified starch.

This study showed a lower pea starch digestibility in dogs at 80.6 ± 2.9 % compared to previous studies reporting that pulse starches are highly digestible in both species with >95% digestibility for lentils and peas when formulated into diets (Carciofi et al. 2008, de-Oliveira et al. 2008). This is also accompanied by lower protein digestibility in dogs which is confirmed by

previous studies (Murray et al. 1999; Carciofi et al. 2008) although in cats our results are slightly higher than previously reported (de Oliveira et al. 2008). The major difference between studies is the processing method used to purify the pea starch and resulting purities of the pea product used. In the current study, air classification (dry processing) resulted in a pea starch that still retained ~15% protein (similar to the lentil and faba bean starch used in this study) and likely retained much of the native, resistant pulse starch structure (see Chapter 3 of this thesis). In contrast, the high digestibility of the other previous studies is likely explained by the use of highly purified (~99% starch), wet-processed pea starch (Carciofi et al. 2008; de Oliveira et al. 2008). All digestibility diets in this thesis and the previous studies used extruded diets, a process that cooks the starch to cause gelatinization, making it more susceptible to digestion (Erayu et al. 2009). In contrast, upon cooling after extrusion, retrogradation can occur and legumes are reported to be more susceptible than cereal starches to undergo this process which would decrease digestibility (Korus et al. 2008). Therefore, either the method of starch purification or retrogradation after extrusion of the digestibility diets contributed more resistant starch and explains our observation of lower starch digestibility of all the pulse starches tested in both dogs and cats. Food with lower *in vitro* digestibility has been linked to lower *in vivo* glycemic responses in humans and recently the same has been reported in dogs (Carciofi et al. 2008) and cats (de-Oliveira et al. 2008) of various starch sources rather than just varying amounts of a single carbohydrate source (Thiess et al. 2004; Elliott et al. 2012).

4.4.2. Weight Control and Basic Health

In dogs, all of the test diets caused some weight loss (a highly desirable property for most pets), while only the modified cornstarch diet caused weight loss in cats. The observed weight changes in both dogs and cats was expected with the modified cornstarch diet due to the

hydroxypropylated cornstarch being less digestible *in vitro* when compared to a regular cornstarch (Chung et al. 2008b, more detail discussed below). Another reason for this weight loss may be due to the fact that the commercial food which the animals were maintained on contained highly digestible whole grain corn and brewers rice, where as our diets contained less digestible pulses. Since increased weight gain is a known risk factor for insulin resistance and diabetes in dogs and cats (Rand et al. 2004) it is important to monitor and keep our pets in the healthy range. Because weight loss with the modified cornstarch diet may have been linked to diarrhea while the pulse starch diets were not, I can recommend that pulse starches be used over other starch sources in dogs, but not cats, to promote weight maintenance.

Basic health after feeding each test diet can be inferred from blood chemistry and complete blood count results. Although some chemistry values were statistically changed with diet, the majority of values were within reference range and thus all cats and dogs can be considered healthy after being fed the test diets. An example was total plasma protein which was low at the end of feeding all test diets in dogs, but still within reference range. While plasma protein can be an indicator of liver and kidney function, it can also indicate dietary protein quality. Regardless, a recent study published in Veterinary Clinical Pathology (Ruaux et al. 2012) states that for total protein in dogs, a population-based reference value is of limited use, considering variation among healthy dogs. In contrast, the cat blood parameters showed higher than reference levels for cholesterol following faba bean diet. Recently a study by Falkeno et al. (2016), showed that cholesterol had high variation among individuals therefore the use of a population-based reference range is limited. Taken as a whole, the blood chemistry and blood cell counts indicate that both cats and dogs were healthy at the end of feeding all test diets.

4.4.3. Glycemic Control and Insulin Sensitivity

Lower GI values in cats than dogs were seen following diet acclimation compared to the GI values produced when the animals were not acclimated to the diet (compare to Chapter 3). This is consistent with reports that cats have carnivorous metabolic adaptations (Kienzle. 1993b; Batchelor et al. 2011), which would be predicted to cause a lower ability to digest starch and transport glucose (Buddington et al. 1991), leading to lower glycemic responses. However, an expected side effect would be diarrhea due to higher colonic starches and sugars (Kienzle. 1993b). Cats have a short colon and a non-functional cecum (Sunvold et al. 1995). Combined with the fact that cats are carnivores, this led to the belief that negligible fermentation occurs in the feline hindgut (Maskell & Johnson. 1993; Pagan. 2011). However, studies have shown that cats in fact do undergo fermentation in the hindgut following ingestion of various fibre sources (Kienzle. 1993b; Sunvold et al. 1995a; Sunvold et al. 1995b; Brosey et al. 2000; Verbrugghe et al. 2009). The degree of fermentation, as shown by the production of volatile fatty acids, is similar to both ruminants and monogastric mammals (Brosey et al. 2000). The high digestibility and low GI responses may be explained by fermentation of the starch sources.

The amount of SGLT-1 in cats has been reported to be sufficient for the amount of carbohydrates that they consume in their natural diet (Batchelor et al. 2011). However, normal dog and cat diets can contain from 30-60% ME from carbohydrates. Previous work done in our lab with pea and rice-based diets showed similar postprandial glycemic responses to both diets in dogs (Adolphe et al. 2015). Work in this thesis found similar results in that we saw no significant differences in terms of glycemic responses among the diets. Our results in cats are also similar to other studies, in which no changes in postprandial blood glucose were observed with either a carbohydrate free diet, or diets containing potato starch or cornstarch (both raw and cooked) (Kienzle. 1994a), or with diets containing cassava flour, brewers rice, sorghum, peas or lentils

(de Oliveira et al. 2008), although both of these were not long-term studies. Considering we saw no significant differences in the major glycemic response measurements, peak, AUC and GI, these may not be the best assessment of metabolic health and instead insulin responses may be better. Changes in insulin responses could be a more sensitive indicator of health status for the long-term and could indicate potential insulin insensitivity which can lead to insulin resistance, diabetes and other associated health complications.

Studies showed changes in insulin responses following a GTT, after chronically consuming diets containing different carbohydrate sources in dogs (Adolphe et al. 2015) or different levels of carbohydrates in cats (Verbrugghe et al. 2010) while the glucose responses showed no significant changes. Pulse diets fed for a long-term period may be able to improve insulin responses, improve insulin sensitivity and lead to less cases of insulin resistance and diabetes in both species. Previous work done in our lab shows that following a 12-week feeding trial of either a pea or rice-based diet, the pea diet showed reduced postprandial insulin responses, although this study was done in obese dogs (Adolphe et al. 2015) whereas in cats, macronutrient content has been reported to have no effect on insulin sensitivity (Leray et al. 2006) even over a nine-month period (Slingerland et al. 2008). Although we see no significant differences in insulin responses in dogs there is a trend for reduced insulin responses following consumption of pulse diets, while in cats, the lentil diet produced a significantly higher insulin response than the modified cornstarch or faba bean diet. As with the glucose responses, many studies that do examine long-term health effects of carbohydrates in dogs and cats examine levels and not different types of carbohydrates, so it is hard to compare our study of different sources to those studies using only one type of carbohydrate. For this reason, longer term chronic studies may be needed to assess metabolic differences to feedings of different carbohydrate

sources, although our study does show promising results which can translate to longer term consumptions.

4.4.4. Influence of Higher Pulse Protein

Pulses are chosen as an alternative carbohydrate source for potentially beneficial reasons, including higher protein content ranging from 17-40%, which is comparable to meat (18-25%) and double that of cereal grains (7-13%) such as corn and rice (Genovese and Lajolo. 2001). Animal protein content in pet foods can be limited due to potential high levels of ash. Therefore, vegetable proteins offer a cheaper, low ash alternative. Human diets high in protein and low in carbohydrate are reported to have health benefits by improving weight loss and markers of cardiovascular risks which would be especially important for human patients with type 2 diabetes (Ajala et al. 2013). There are also contradictory studies reporting that high protein diets for type 2 diabetic humans lead to worsening of coronary artery disease (Fleming et al. 2000). Similarly, with dogs, it has been reported that high protein and moderate carbohydrate diets provide blood glycemic control and are therefore beneficial for diabetic dogs (Elliott et al. 2012) and cats (Frank et al. 2001), but this does not mean that all animals have to be on these diets. The results from this study highlight an important factor when choosing a low GI carbohydrate for pet food, in that not all of these carbohydrates are the same. Pulses contain bioactive substances which are often labelled as antinutritional factors (Salunkhe. 1982), that have been reported to have both beneficial and non-beneficial effects (Champ. 2002). Further research into health benefits of different pea products for pet foods would benefit not only our pets, but humans as well and pulse producers.

4.4.5. Toxic Glucose Metabolite, Methylglyoxal (MG), and Oxidative Stress

Hyperglycemia can lead to the formation of toxic glucose metabolites, including methylglyoxal (MG). MG is a dicarbonyl metabolite produced not only from glucose, but other energy substrates (protein and lipid) when present in excess amounts and can also be found in food items (Ankrah et al. 1999). Patients with type 1 and type 2 diabetes have elevated blood MG levels (McLellan et al. 1994; Wang et al. 2007) where MG is reported to interfere with insulin action, hindering glucose disposal and potentially playing a role in insulin resistance (Jia et al. 2006). MG is also a precursor for the production of advanced glycation end products (AGEs) and leads to increases in oxidative stress (Desai et al. 2010). The results of the current study showed that consumption of pulse diets versus a modified cornstarch diet over a six-week period does not affect postprandial MG levels in either species. Postprandial MG levels, to our knowledge, have never been measured in cats and dogs prior to this study, other than work previously done in our lab in beagles (Adolphe et al. 2012). Work in humans (Masterjohn et al. 2012) reported similar postprandial increases in MG after oral ingestion of simple, but not complex starches. Based on results of the current study, we can conclude that cats also exhibit postprandial increases in MG after glucose ingestion. However, complex carbohydrates or whole diets caused MG levels to drop below fasting levels (Chapter 3 of this thesis and current study). In both cats and dogs, six weeks of feeding pulse-containing diets did not change postprandial MG responses. It has been suggested that glycolysis is not the main source for plasma MG production, but rather an interaction between glucose and protein (Kalapos. 2013). In fact, protein glycation and aminoacetone degradation are the major and minor sources of plasma MG under normal metabolic conditions in humans. The natural diet of carnivorous cats would normally contain higher levels of protein, so potentially cats may have developed, over time, a more sufficient mechanism for detoxification of MG, leading to their proficiency at decreasing

postprandial MG levels. A carnivorous diet has also been suggested to protect against carbonyl stress by increased consumption of a protective agent, carnosine (Hipkiss. 2005) which could be why we see decreases in postprandial MG levels in cats only. This interesting species difference is an area worthy of further exploration to help us better understand the development of diabetes and how to better protect against it in dogs and humans.

MG is metabolized via the glyoxalase system (glyoxalase 1 & 2) into D-lactate (Kalapos. 1999). MG and its metabolite D-lactate, have been measured in the same individual and have been shown to both be increased in diabetic patients compared to normal non-diabetic patients (McLellan et al. 1994) but that only D-lactate, and not MG, had a positive correlation with blood glucose levels. The next step in this research would be to analyze for D-lactate and glyoxalase 1 & 2 concentrations of which are involved in detoxification. I predict that since the cats are decreasing their postprandial levels compared to pre-feeding levels, and compared to the dogs, that they may be more efficient at metabolizing the MG into D-lactate. One could argue that I saw decreases in cat MG postprandial levels because they initially have a higher level of MG levels. However, results of this study do not support this hypothesis since baseline MG values ranged from 0.69-0.82 μM in dogs, while cats ranged from 0.54-0.90 μM. We can see that both species have similar baseline levels, which is actually lower than what has been reported in healthy humans at 1.4 μM (Jia et al. 2006).

MG is thought to increase oxidative stress (Desai et al. 2010), supported by the observation that there was a positive correlation in humans between oxidative stress and glycemic load (Arikawa et al. 2015). Nitrotyrosine is a well-established marker in plasma and tissue of oxidative stress, produced by oxidative damage to proteins by peroxynitrite. Previous studies reporting levels of nitrotyrosine in dogs only comes from our lab (Adolphe et al. 2012), with

levels comparable to that observed in the current study in cats. However, the levels in the current dog study are ~10-fold lower and more similar to other reports in dogs with tumors or infections (Nemec et al. 2013). This discrepancy is likely due to the fact that the current study used fasting levels and normal-weight dogs, while our previous study measure nitrotyrosine at 60 minutes postprandial in obese dogs (Adolphe et al. 2012). Humans were reported to show postprandial increases in plasma nitrotyrosine following consumption of a high GI carbohydrate, white bread, and a glucose control (Dickinson et al. 2008). While this current study did not examine postprandial changes in nitrotyrosine after acute feedings, chronic feeding of diets with 30% modified cornstarch versus pulse starches did not alter fasting levels of oxidative stress in both cats and dogs. Why cats showed 10-fold higher fasting oxidative stress is unclear but is an interesting species difference and may show a link with postprandial MG levels. MG is known to be detoxified using GSH (Kalapos. 1999), therefore this antioxidant may be being used in the glyoxalase system, leaving less GSH available in the postprandial period in cats for detoxifying ROS, leading to increased oxidative stress.

4.4.6. Methylglyoxal (MG), Oxidative Stress, and Cardiovascular Health

There has been a clear link established with MG concentrations and oxidative stress in numerous cell types *in vitro*, but in terms of *in vivo* it is more of an association (Desai et al. 2010). Increases in oxidative stress biomarkers (Sena et al. 2012) and decreases in antioxidant levels (Dhar et al. 2013) have been shown in both MG and fructose treated rats. These increases in MG and oxidative stress have also been shown to decrease NO dependent relaxation (Sena et al. 2012) and increased blood pressure (Dhar et al. 2013) which can lead to reduced endothelial function and reduced cardiovascular health. To our knowledge, no other studies have reported postprandial MG levels in dogs or cats except for a previous study done in our lab. Adolphe et al.

(2012) reports an increase in postprandial MG levels following consumption of a simple carbohydrate and this was also associated with reduced flow-mediated dilation. I found similar results in that feeding of a simple carbohydrate (glucose) increased postprandial MG levels in both species, but when fed a whole diet these increases were only seen in dogs and following the lentil diet in cats. Despite these species differences in postprandial MG I did not see any cardiovascular effects that specifically warrant any further research. Pulse consumption has been shown to have positive effects on cardiometabolic health by decreasing risk factors such as blood pressure (Jayalath et al. 2014) and obesity in humans (Kim et al. 2016). Long-term consumption of high GI foods has been shown to increase cardiovascular disease risk in women, but interestingly not men (Dong et al. 2012). Therefore, long-term consumption of pulses in dogs and cats may induce the same cardiometabolic health benefits including blood pressure which is associated with increased MG levels.

4.4.7. Conclusion

The results from this study showed that the addition of pulses to both dog and cat diets have health benefits in terms of controlling glycemic and insulinemic responses and offering a low GI diet. Despite this, the insulin responses to pulses in cats are not equal, which is why not only the level of carbohydrate but the type used is important for food formulations. Unlike pea and faba bean starches, lentil starch in cats led to decreased insulin sensitivity, suggesting that use of this starch in cat diets would not be as healthy long-term.

Results of the current digestibility study in cats indicate that carbohydrates are being digested without fecal excretion, but yet are producing minimal increases in blood glucose. Moreover, this digested glucose is not being shunted into producing the toxic glucose metabolite, MG. Therefore, our results provide supporting indirect evidence that cats use fermentation of

digested, but not absorbed carbohydrate in the hindgut, but this requires confirmation with further experiments. If true, then pulse starches sources which are naturally higher in resistant starches and fibre would better promote this fermentation and be more suitable for dietary starch sources in cat foods than highly digestible, high GI starch sources.

5. NON-PUBLISHED DATA-VASCULAR ULTRASOUND RESULTS OF BOTH DOG AND CAT FROM ACUTE AND LONG-TERM FEEDING TRIALS.

This data chapter is the vascular ultrasound measurements from both the acute feeding study (Chapter 3) and long-term feeding study (Chapter 4) from both dogs and cats. This data allows us to evaluate any vascular changes in the diameter of the arteries due to consumption of either the starches or the formulated diets of which we have data showing results for both short term and long-term consumption.

This chapter will not be published.

Jennifer Briens did 100% of the animal work, data collection and analysis for cardiovascular parameters. Dr. Lynn Weber contributed through scientific input and editing of writing. No other co-authors contributed to this chapter.

5.1. Introduction

It is widely accepted that cardiovascular function can both be positively and negatively affected by nutrition in humans, but less has been studied when it comes to companion animals about this topic. Cardiovascular parameters were measured for this thesis as a marker of endothelial health. Hyperglycemia has been shown to negatively affect endothelial function, with acute hyperglycemia impairing macrovascular, but not microvascular endothelial function in humans (Loader et al. 2015). Another study showed evidence that in humans, increased glucose variability, rather than prolonged hyperglycemia causes more endothelial damage (Ceriello et al. 2008). For this reason, if our pets are consuming meals containing high GI carbohydrates they could be at risk for reducing endothelial health and developing cardiovascular disease. This is of concern for our pets considering more and more dogs and cats are coming into clinics with diabetes (Hoenig. 2012; Rand et al. 2004). If hyperglycemia causes endothelial dysfunction and an increase in animals are presenting with diabetes, then there is also the risk of increased cases of cardiovascular disease.

Nitric oxide (NO) is an important vasodilator involved in many endothelial-dependent functions including maintaining normal circulation. NO levels can be decreased by reactive oxygen species (ROS) which can be found in numerous conditions such as atherosclerosis, hypertension and diabetes (Kojda & Harrison. 1999). This can be reversed by the addition of an antioxidant such as glutathione (Prasad et al. 1999), to help with increased endothelial function. Although NO was not reported as part of this chapter it is important for endothelial health and was measured and reported as part of the long-term study (Chapter 4 of this thesis). Reduction in NO can lead to impaired vasodilation which would affect arterial dilation and reduce the difference in our measurements.

5.2. Materials and Methods

5.2.1. Ultrasound Analysis

Ultrasound measurements were taken using a SonoSite M-Turbo ultrasound machine with a vascular transducer L38x/10-5 MHz (SonoSite Canada Inc., Markham, Ontario). All measurements were performed by one individual to reduce discrepancies. Arterial visualization was used as a measurement of endothelial health, by assessing images captured during systole and diastole. Pulsed wave Doppler, video clips and still images were taken at time 0 (pre-feeding) and 60 minutes postprandial in each animal following each treatment. For analysis, three measurements were taken at systole and diastole each, of which the difference was used to evaluate arterial stiffness. The median artery in the forelimb was visualized in the dogs and the abdominal aorta was visualized in the cats. M-mode was used to capture a video clip of the artery from which still images were captured at systole (max. arterial diameter) and diastole (relaxed arterial diameter) for analysis using Image-Pro Express 6.0 (Media Cybernetics Inc., Silverspring, USA). Doppler ultrasound was used to capture pulse wave blood velocity.

5.2.2. Statistical Analysis

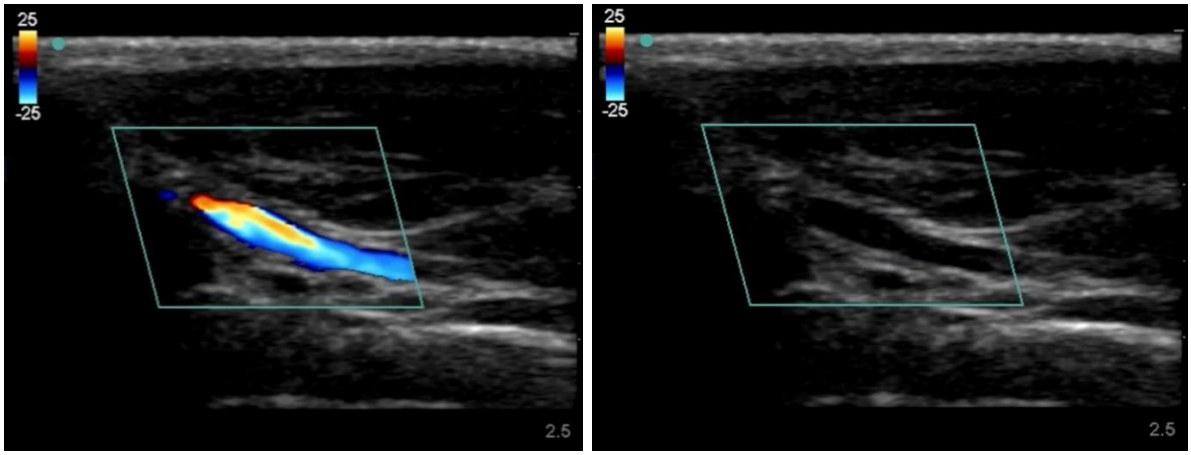
Data (means ± SEM) were analyzed by IBM SPSS version 20.0 (International Business Machine Corp., USA). Related samples t-test was used to determine differences for cardiovascular measurements. One-way repeated measures ANOVA was used to determine differences among treatments. Mauchly's test was used to test for sphericity. Post hoc analysis was performed using Fisher's least significant difference (LSD) to determine any pairwise differences. A $p<0.05$ was considered significant. Independent samples t-test were used to determine species differences within a treatment. Levene's test was used to determine equal variances.

5.3. Results

The acute results are summarized in Table 5.1 and 5.2 showing values from the single feedings of the starch sources and whole diets respectively. Average baseline diameter taken from time 0 pre-feeding for cat abdominal aorta was 0.375 cm and average baseline diameter for dog median artery was 0.187 cm. Following the single feedings of the starch sources we see no significant differences between pre-feeding measurements and 60 minutes postprandial in both species. Despite the lack of significant differences, we do see a trend for a decrease in arterial distensibility from pre-feeding to postprandial following consumption of the glucose control, tapioca starch and rice flour in dogs, all of which produced high glycemic responses. In cats, this association is less clear with decreases in arterial diameter distensibility following consumption of rice flour, potato starch and faba bean starch. This would be expected for rice flour and potato starch since they produced high GI values, but faba bean produced one of the lowest GI values in cats. Following the acute feedings of the whole diets we see that only the modified cornstarch diet produced a significantly larger diameter change at 60 minutes postprandial in the dogs ($p = 0.042$). All other results in both the dogs and the cats showed no significant differences between the pre-feeding (time 0) and 60 minutes postprandial results. We also don't see any trends similar to what we saw for single feedings of the starch sources alone.

Looking at the long-term feeding trial results, the ultrasound arterial diameter measurements are summarized in Table 5.3. A significant decrease was observed in arterial diameter following the OGTT in dogs after consuming the pea diet for six weeks ($p = 0.050$). Other than this result, no significant differences were observed between time 0 and time 60 for any of the other treatments in both dogs and cats. Again, we see no trends occurring following consumption of the diets that could be linked to any metabolic responses.

DOG



CAT

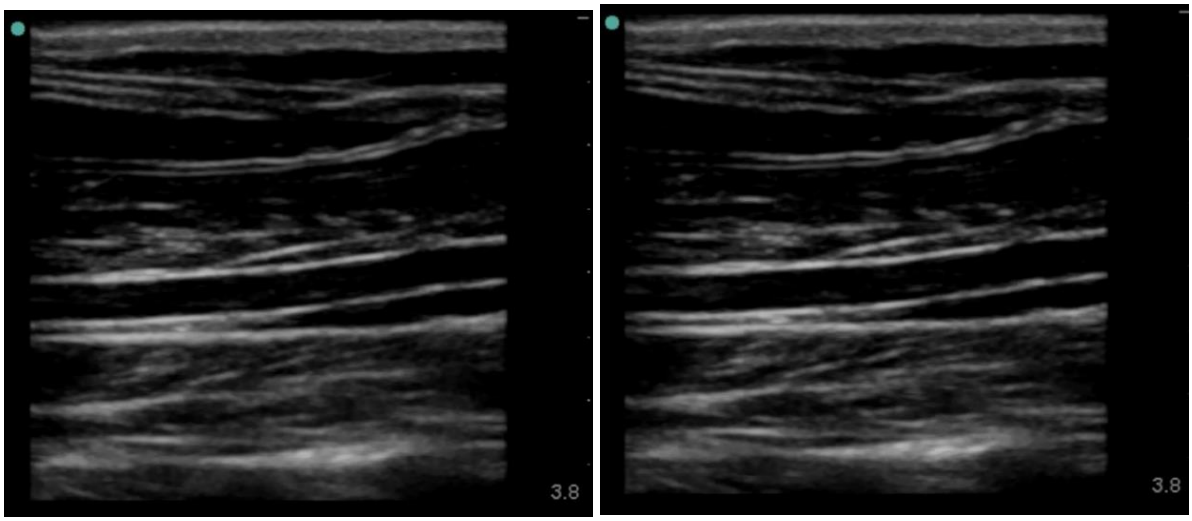


Figure 5.1 Ultrasound M-mode was used to capture a video clip of the artery from which still images were captured at systole (max. arterial diameter- left image) and diastole (relaxed arterial diameter-right image) for both dog (top) and cat (bottom).

Table 5.1 Ultrasound analysis of median artery (dog) and abdominal aorta (cat) showing the baseline and change in arterial diameter with each pulse (cm) at time 0 (pre-feeding) and 60 minutes postprandial following single feedings of starches and glucose control.

Time (min)	Baseline (cm)	Glucose	Tapioca	Wheat	Rice	Unmod Corn	Pea
DOG	0.187						
0		0.028±0.005	0.029±0.004	0.022±0.002	0.026±0.005	0.024±0.004	0.025±0.004
60		0.025±0.004	0.027±0.003	0.024±0.004	0.025±0.004	0.026±0.004	0.027±0.002
CAT	0.375						
0		0.029±0.003	0.029±0.005	0.036±0.003	0.035±0.004	0.030±0.002	0.029±0.004
60		0.029±0.003	0.034±0.005	0.036±0.006	0.031±0.004	0.031±0.002	0.032±0.004
Time (min)	Baseline (cm)	Mod Corn	Lentil	Faba bean	Potato	Mod Corn	
DOG	0.187						
0		0.023±0.002	0.024±0.003	0.025±0.003	0.023±0.002	0.024±0.003	
60		0.026±0.002	0.030±0.004	0.031±0.004	0.023±0.003	0.030±0.004	
CAT	0.375						
0		0.028±0.002	0.032±0.005	0.032±0.004	0.034±0.002	0.032±0.005	
60		0.031±0.003	0.032±0.006	0.025±0.002	0.026±0.005	0.032±0.006	

Values are mean ± SEM; n = 8 (dogs), n = 8 (cats), in duplicate for glucose. For each time point three relaxed diameters (cm; min) and three dilated diameters (cm; max) were measured for each animal and the difference is the value reported. Time 60 values with a * are significantly different than the time 0 for the same treatment within a species; $p < 0.05$, related samples t-test used to evaluate differences. Unmod corn = unmodified cornstarch; mod corn= modified cornstarch.

Table 5.2 Ultrasound analysis of median artery (dog) and abdominal aorta (cat) showing baseline and difference between relaxed and dilated arterial diameter (cm) at time 0 (pre-feeding) and 60 minutes postprandial following single feedings of whole formulated diets and glucose control.

Time (min)	Baseline (cm)	Glucose	Modified Corn diet	Pea diet	Lentil diet	Faba bean diet
DOG	0.187					
0		0.028±0.005	0.024±0.004	0.026±0.003	0.024±0.004	0.024±0.004
60		0.025±0.004	0.030±0.003*	0.024±0.003	0.034±0.005	0.024±0.004
CAT	0.375					
0		0.029±0.003	0.031±0.003	0.030±0.003	0.030±0.005	0.037±0.002
60		0.029±0.003	0.033±0.004	0.030±0.002	0.031±0.003	0.028±0.005

Values are means ± SEMs; n = 8 (dogs), n = 8 (cats), in duplicate for glucose. For each time point three relaxed diameters (cm; min) and three dilated diameters (cm; max) were measured for each animal and the difference is the value reported. Time 60 values with a * are significantly different than the time 0 for the same treatment within a species; $p < 0.05$, related samples t-test used to evaluate differences.

Table 5.3 Ultrasound analysis of median artery (dog) and abdominal aorta (cat) showing difference between relaxed and dilated arterial diameter (cm) at time 0 (pre-feeding) and 60 minutes postprandial following six weeks of feeding formulated diets with 30% starch inclusion of either modified cornstarch, pea starch, faba bean starch or lentil starch.

Time (min)	Modified Corn Diet	Pea Diet	Faba bean Diet	Lentil Diet
GTT				
DOG				
0	0.023±0.005	0.027±0.001	0.023±0.004	0.023±0.002
60	0.022±0.003	0.023±0.001*	0.022±0.004	0.021±0.003
CAT				
0	0.023±0.002	0.026±0.002	0.026±0.002	0.026±0.002
60	0.024±0.002	0.026±0.001	0.027±0.002	0.029±0.002
Diet Feeding				
DOG				
0	0.024±0.004	0.023±0.001	0.024±0.002	0.025±0.003
60	0.028±0.005	0.024±0.004	0.024±0.003	0.019±0.003
CAT				
0	0.020±0.002	0.025±0.002	0.026±0.002	0.027±0.002
60	0.024±0.002	0.022±0.002	0.024±0.001	0.023±0.002

Values are mean ± SEM; n = 8 (dogs), n = 9 (cats; pea, lentil and faba bean starch), n = 8 (cats; modified cornstarch). For each time point three relaxed diameters (cm; min) and three dilated diameters (cm; max) were measured for each animal and the difference is the value reported. Time 60 values with a * are significantly different than the time 0 for the same treatment within a species; $p < 0.05$, related samples t-test used to evaluate differences.

5.4. Discussion

Ultrasound analysis can be an efficient way of determining arterial health, due to imaging of pulsatility. From this analysis, we can determine any changes to endothelial cells, which can lead to hypertension. There are various methods of visualizing arteries and measuring blood pressure. Non-invasive indirect methods, such as Doppler are commonly used in veterinary clinics for measurement of arterial health. I evaluated the difference in arterial diameter between dilation and relaxation as a measurement for endothelial health, and then compared the changes seen from pre-feeding to 60 minutes postprandial to determine if diet was playing a role in causing endothelial changes following both acute feeding of starch sources and whole diets, and long-term feedings of the whole diets. Healthy arteries should dilate and return to a relaxed position with each pulse of blood. A reduction in diameter changes indicates arterial stiffening. In a study done on healthy dogs, they showed that brachial diameter changes were on average 0.13-0.28 mm following flow mediated dilation (FMD). FMD is a widely used method for determining endothelial health, and although I did not conduct this for our study, previously in our lab it was shown that a simple carbohydrate feeding reduced postprandial FMD compared to complex carbohydrate sources (Adolphe et al. 2012). To our knowledge this is the only study that has measured postprandial arterial diameter changes in healthy dogs following consumption of either a simple or complex carbohydrate source. Although we see a significant increase in arterial diameter following consumption of the modified cornstarch diet in the acute study in dogs, I would not suggest this as a biologically relevant finding. These results were also not corroborated in the long-term study, where we only saw a decrease in arterial diameter in dogs following consumption of the pea diet.

One of the challenges associated with conducting ultrasound analysis on the dogs and cats was that I did not use sedation to steady the animals. Accuracy of measurements is important for determination of changes over time, for this reason the same spot should be used on each animal for each measurement. With the lack of sedation, and even with regular handling of the animals, it was difficult to accurately take a measurement on the exact same spot. Another challenge experienced during this study was maintaining the same position for the animals during the repeated measurements. Some positions may lead to occlusion of the artery due to the recumbent position the animals are in, which could affect the results. For this reason, this data is included in a non-published section. In other studies that have assessed FMD using ultrasonography, they have reported poor repeatability and high variation within one dog and among dogs, which may be due to movement artifacts, or suboptimal image quality (Puglia et al. 2006; Jones et al. 2010).

6. OVERALL DISCUSSION

6.1. Summary of Conclusions

The research done for this thesis examined dog and cat metabolic responses to different carbohydrate sources as a single ingredient and in whole formulated diets, used in both an acute and chronic feeding study. The main research objective of this study was to examine species differences in carbohydrate metabolism and to determine if pulse starches provide an alternative carbohydrate source that improves metabolic health. The results from this thesis showed that pulse starches do provide a healthy alternative carbohydrate option compared to corn and rice in both dogs and cats. Therefore, the overall research hypothesis can be accepted that pulse starches do provide a low GI carbohydrate option for pet food that can provide long-term health benefits in dogs and cats.

The research done for this thesis is a continuation from the previous work done in our lab which determined that using peas as a starch source provided metabolic health benefits compared to rice when used in dog foods. Literature is sparse in reporting GI values of different carbohydrate sources using appropriate methodology in dogs, but absent in cats. This thesis addressed this knowledge gap previously acknowledged by both industry and academia (Aldrich. 2013). The following is a short summary of the most significant findings from each of the studies in this thesis:

1. The acute study (Chapter 3) showed that pulses provide a low GI starch source in both species. Moreover, cats can utilize carbohydrates and control postprandial blood glucose levels as shown by the very low GI values and high starch digestibility in this species.

2. The acute study (Chapter 3) showed that formulation into complete diets increased the GI compared to the starches alone. This is likely due to processing, but pulse starch-containing diets nonetheless produced low GI values for both species.
3. The acute study (Chapter 3) showed that postprandial MG responses are linked to the GI values in dogs. In contrast, in cats there was no link, and instead, MG tended to decrease in the postprandial period after feeding all carbohydrates tested except pea and glucose.
4. The six-week feeding study (Chapter 4) showed that low glycemic indices for the pulse diets were maintained after six weeks of feeding, especially in cats.
5. Chapter 4 showed that six weeks of feeding diets with different starch sources (modified cornstarch versus pulse starches) had no effect on glycemic responses in both species.
6. Chapter 4 showed that six weeks feeding of diets with 30% modified cornstarch, despite its low digestibility and GI in dogs, decreased insulin sensitivity (higher peak insulin following a, while diets containing pulse starch did not change insulin sensitivity. In contrast, six weeks of feeding diets with both modified corn and lentil starches in cats led to decreased insulin sensitivity (higher insulin peak for corn, higher insulin AUC for lentil), suggesting that use of these starches in cat diets would not be as healthy long-term.
7. Chapter 4 showed that after six weeks of feeding the test diets, the link between GI and postprandial MG responses observed in the acute feeding study (Chapter 3) in dogs was no longer evident (Chapter 4). Interestingly in the cats after six weeks of feeding and following the acute diet test, the lentil diet produced the highest AUC insulin value which corresponded with the highest change in MG levels, but this link is not clear.

The results from the acute study (Chapter 3) showed that there are species differences in carbohydrate handling, but these differences are not what is assumed in the literature in terms of the carnivorous adaptations of cats (MacDonald et al. 1984; Farrow et al. 2002; Zoran. 2002). Possibly through domestication over the years, cats have adapted from a high protein and fat diet to be able to digest and utilize carbohydrates as a source of energy and avoid high blood glucose excursions. Dogs, being omnivores, follow our hypothesis of how they should digest and metabolize carbohydrates. In contrast, cats led me to reject our hypothesis that they would handle carbohydrates poorly compared to dogs and largely disagreeing with ideas put forth in extensive literature about cat carbohydrate metabolism (Kienzle. 1994a; Kienzle. 1994b; Zoran. 2002; Appleton et al. 2004; Rand et al. 2004; Hewson-Hughes et al. 2011).

Not only does this research have implications for the health and wellbeing of our pets but this could expand the market for pulses in the pet food industry. Peas, lentils and chickpeas are becoming more and more common in pet foods, but the scientific reasoning behind their addition in diets is lacking. The research presented in this thesis provides support for the marketing of pet foods with pulses as the carbohydrate source to as having a low GI. Overall the results from this study showed that pulse-based diets are a low GI alternative carbohydrate option for pet food in both dogs and cats, but that not all pulses are equal. Moreover, dogs and cats do not metabolize pulse diets the same. Despite species differences in responses to feeding pulse-containing diets between cats and dogs, both species showed similar responses to feeding simple carbohydrate (glucose). In response to an oral glucose challenge, both dogs and cats increased postprandial blood glucose and increased MG production more than feedings of complex carbohydrates, and interestingly in cats, I see decreases of this metabolite which warrants further research.

6.2. Strengths and Limitations

A strength of this study is that I am making the comparison between species, dogs and cats. This study highlights some important differences between the two species and may change some of what we believe about metabolism in cats. Despite their carnivorous ancestry, this thesis shows that they are able to utilize 30% inclusion of carbohydrate sources in diets, but the upper limit is unknown. Combined with the indirect evidence that cats may be capable of significant hindgut fermentation, this opens the door to examine higher inclusion rates of pulses in both cat and dog diets.

A few more strengths of this study are that I used cephalic vein catheterization for blood collection and that the animals were challenged with an oral, not intravenous, glucose test. Most studies referenced in this thesis use jugular vein catheterization for blood collection. Reasons to use the jugular include being able to obtain larger volumes of blood and that this is a common vein used in clinical settings for blood analysis. However, the cephalic vein was chosen for this thesis due to the amount of times I was going to have to repeatedly place an intravenous catheter (average of 16 times for acute study and eight for chronic study, not including replacements when catheters became clotted mid-test). Placing a jugular catheter this many times is not ethical, is very uncomfortable for the animals and is worsened by the fact that during testing, they are kept confined in the testing room for most of a day without any sedation. Another reason for using the cephalic vein stems from human studies where forearm vein or finger capillary are used to obtain blood samples. To determine glycemic responses during an OGTT, humans drink a glucose solution. Oral glucose testing is better than IV glucose testing at estimating glucose handling when testing for diabetes in humans (Olefsky et al. 1973). Conducting the GTT using oral administration, glucose is exposed to all enzymes of the gastrointestinal tract similar to what is necessary for determining GI values for complex starch and whole diet testing. If I had

administered the glucose solution intravenous, glucose bypasses enzymes and transporters that can affect its availability and kinetics of glycemic responses are greatly altered. Thus, when conducting feeding trials, oral glucose feeding reduces discrepancies between methods.

GI has been known as the standard for classifying the ability of a carbohydrate source to increase blood glucose. Recently it has been suggested that the use of glycemic load (GL) may be a better predictor for postprandial glycemic responses, (Bao et al. 2011). Glycemic load takes into account both the GI of the carbohydrate and the amount of NFE in grams. GL is being used more and more in nutritional epidemiological studies in humans. In fact, glycemic load has more real-world relevance for both humans and animals since both do not consume only one food product per meal. Carbohydrates vary in their effects on glycemic and insulinemic responses, thus the whole meal must be considered, not just a single starch. GL has been shown to be a better predictor of postprandial glycemic and insulin responses than carbohydrate content alone (Bao et al. 2011). Numerous chronic diseases have positive associations with high GL diets, including type 2 diabetes, heart disease, breast cancer, and gallbladder disease (Barclay et al. 2008). With human nutrition research going in this direction, we should follow suit in the pet nutrition research area. This thesis has set the stage by determining GI values in dogs and cats, but follow-up studies should use glycemic load to better interpret findings, especially if multiple carbohydrate sources are used in pet food formulations.

One potential limitation of this study was that I did not use any form of sedation for our procedures, such as during placement of the intravenous catheter, ultrasound, blood pressure measurement or force-feeding of liquid starch solutions. These are stressful events for any animal, particularly the cats. Stress alone can lead to increases in heart rate, blood pressure and resting blood glucose levels (Koeppen & Stanton. 2017). However, if sedatives were used, the

sedatives themselves have the potential to also adversely affect or confound each of these same physiological parameters. In order to minimize the effects of stress in the experiments in this thesis, animals were acclimated to the procedures with positive reinforcements for a month before beginning the study. Despite this, slightly elevated fasting (pre-feeding) blood glucose levels were observed in some, but not all of the cats (3.6-8.5 mmol/L) during glycemic testing, whereas this was not observed in the dogs (3.6-5.2 mmol/L). Reference ranges for dog blood glucose levels are reported to be 4.2-6.6 mmol/L and cats reference range is 3.3-6.7 mmol/L (Merck Manual. 2017). Even with acclimation, inserting an intravenous catheter is still uncomfortable for the animals, no matter how much practice the tester has and will never be an enjoyable procedure. Moreover, cats are not an easy species to work with and even with acclimation, are very temperamental, not always willing to sit still and do not like to be restrained. The previously mentioned increases stress levels in cats, making their baseline blood glucose, blood pressure and heart rate the most suspect compared to that obtained in the dogs. I have realized, much like humans, that cats have good days and bad days which ultimately could affect our results.

6.3. Future Work

The results from this study showed that both dogs and cats can utilize pulse carbohydrate sources in their diets at an inclusion rate of 30%, without experiencing high glycemic excursions. At 30% inclusion, both dogs and cats efficiently digested and metabolized the pulse starches, using them as an effective energy source. A next step in this research could be to perform a pulse starch tolerance test, formulating and testing diets with increasing inclusion rates above 30%. Carbohydrates are necessary in pelleted diets due to its binding properties which help with forming nice pellets, therefore total exclusion of carbohydrate is not suggested. Moreover, high

protein diets, although viewed as desirable by a segment of the pet food industry and owners, is too costly, is not sustainable (Deng & Swanson. 2014). From this we could determine at what percentage the carbohydrates are no longer efficiently digested, or at what point blood glucose and insulin responses are negatively affected. However, high inclusion rates of any nutrient can lead to underutilization, waste of ingredients, and potential high blood glucose or insulin if there is excess carbohydrate, so a balance must be found. Carnivores such as cats have higher requirements and consume higher levels of protein and fat in their natural diets than omnivores (NRC. 2006; Eisert. 2011). Pulses have levels of proteins that usually range between 17-30% (Boye et al. 2010). Addition of pulses in cat food could be a more sustainable way to increase protein content in their diet since pulses are cheaper than an animal meat alternative. If pulse starches such as that tested in this thesis, were included at moderate levels, this would help increase the protein content of the diet, while maintaining lower blood glucose levels.

The insulin results from the single feedings of the starches alone open some questions about effects of the pulses on insulin in cats. For this thesis I have focused on the carbohydrate content of the pulses, but the effects of the protein content may warrant some interesting results. It has been suggested that in cats, unlike humans and dogs, the proteins may be the main contributor to changes in metabolic parameters versus carbohydrates. Evaluation of the protein content of pulses for pet food could be a direction to go next for future research and evaluating species differences to the proteins would be beneficial.

As mentioned previously in the long-term study, not all pulses seem to be the same in either species. In dogs, peas and lentils appear to be the better options. Although there were no significant differences seen between the pulse diets for glycemic, insulinemic and MG responses the pea and lentil starch diets produced lower levels of MG during the single diet feeding

following six weeks of diet consumption. In cats, peas and faba beans were found to be the better two starch sources based on lentil producing the highest insulin response, highest GI, and highest increase in MG compared to the other pulse diets following a single feeding test after six weeks of feeding. This opens questions as to why metabolic responses to peas, lentil and faba bean starches are not the same. Questions remain whether differences are due to other bioactive compounds present in pulses, differences in starch structure or some other factors. There is also the potential to combine different pulses in pet food diets. In humans there are known benefits of combining grains and pulses. Pulse proteins are high in lysine but low in tryptophan while grains are higher in sulfur containing amino acids (Mudryj et al. 2014). Therefore, combining two different pulses or exploring combining a pulse with a grain may give a balanced, more complete amino acid profile (Food and Agriculture Organization. Accessed May. 2017; Rebello et al. 2014). This could be especially beneficial for cats since they need higher levels of proteins, as long as the low GI that the pulses offer is maintained. Research into this as a pet food option needs further research.

The results of this study are contradictory to what has been previously reported in the literature regarding feline carbohydrate metabolism. One of the most interesting findings was that cats had decreased postprandial MG levels following feedings of complex carbohydrates and whole diets. Further research in this area is needed to understand the mechanisms cats use to metabolize and detoxify this metabolite. Next steps would be to examine D-lactate, the metabolite of MG. If these levels are higher in cats than dogs, this would indicate that cats have enhanced detoxifying mechanisms. We could also look into analyzing the glyoxalase system by using PCR. It would be beneficial to look at feedings of whole diets (high GI vs low GI) during a chronic feeding period as this would represent a pet's life consuming the same diet on a daily

basis. Comparing dog and cat results will demonstrate if there are species differences in MG metabolism and detoxification.

This study was done using one breed of dogs and domestic shorthair cats. To expand on this study, it would be beneficial to evaluate different breeds of dogs. Beagles are used in nutrition studies because they have a good temperament and are not picky eaters, which is why there were no palatability issues with the beagles. Other dog breeds can tend to be picky when it comes to their food, and there are differences when it comes to large and small dog breeds in terms of metabolism and disease risk (Fleischer et al. 2008). For this reason, it would be beneficial to evaluate long-term health benefits of pulse diets in various breeds, and also increase the number of animals used in a study. Finally, an epidemiology study using pets living normally in owner homes would be the ultimate confirmation of benefits. In the real world, pets are exposed to a highly variable conditions and health status. An epidemiology study could use a feeding period with increased length as well as larger sample size to better determine long-term health effects.

The pet food industry, similar to human nutrition, is always looking for new functional food ingredients that provide nutritional benefits, are sustainable and cost effective and can improve palatability of food. One of these ingredients is fermented yeast. Using fermented products in pet foods helps to increase palatability since dogs and cats show a preference for umami flavours. Fermented foods have been suggested to be beneficial in terms of their vitamin, mineral, fibre, and antioxidant content (Rivera-Espinoza & Gallardo-Navarro. 2010). Combining this knowledge with what I have presented in this thesis, the use of a fermented pea product in pet food would be a very interesting area to explore. One of the main challenges I had with the cats was palatability of our test diets. The cats avoided the first diet (digestibility diet) without any palatant, so the formulations were revised to included fish oils and palatants. Fish oils have been

shown to be beneficial in terms of decreased risk for diabetes and cardiovascular disease in humans (Harris et al. 1988). However, for the purposes of our studies, adding too much fish oil into the test diets was not desirable since this could have overshadowed any health benefits observed with using pulse starches. However, this is something that should be explored in future studies.

6.4. Final Conclusion

The results from these studies not only could have positive impacts for both animal and human health and also benefit the Saskatchewan and Canadian agriculture economy. In 2016, Saskatchewan exported over 5 million tonnes of pulses equalling around 3.5 billion, with the vast majority of that being peas and lentils (Saskatchewan Pulse Growers. 2017). With many advances in pet nutrition our animals are living longer which also means increases cases of chronic disease such as obesity and diabetes. Pulse diets may help to decrease incidence of these diseases by providing glycemic control. This will be beneficial to pet owners by reducing costs of veterinary bills. Peas are already a common ingredient in dog food, but this research opens up the possibility to using other pulse grains in food formulations not only for dogs but cats as well. This research will help to expand the market for pulse crops and increase the demand for pulses in the pet food industry which will have benefits for the Canadian pulse industry. Not only will pulses benefit the Canadian economy, they are sustainable ingredient. Protein sources for pet foods usually come from secondary products of human food. Therefore, to minimize competition the pet food industry is continually looking for alternatives (Deng & Swanson. 2014). Pulse crops would be a beneficial addition into pet food diets as plant-based protein sources, which would allow pet food companies to decrease the amounts of animal proteins. This would increase

sustainability and decrease the carbon footprint of the pet food industry (Deng & Swanson. 2014).

REFERENCES

Abebe, W., Collar, C., Ronda, F. 2015. *Impact of variety type and particle size distribution on starch enzymatic hydrolysis and functional properties of tef flours.* Carbohydrate Polymers. Vol. 115(22): 260-268.

Adolphe, J.L., Drew, M.D., Huang, Q., Silver, T.I., Weber, L.P. 2012. *Postprandial impairment of flow-mediated dilation and elevated methylglyoxal after simple but not complex carbohydrate consumption in dogs.* Nutrition Research. Vol. 32: 278-284.

Adolphe, J.L. 2013. *Acute and chronic effects of low versus high glycemic index carbohydrate sources on metabolic and cardiovascular responses in lean and obese dogs.* PhD Thesis. University of Saskatchewan.

Adolphe, J.L., Drew, M.D., Silver, T.I., Fouhse, J., Childs, H., Weber, L.P. 2015. *Effect of an extruded pea or rice diet on postprandial insulin and cardiovascular responses in dogs.* Journal of Animal Physiology and Animal Nutrition. Vol. 99(4): 767-776.

Agriculture and Agri-Food Canada. 2012. *Consumer Trends: Pet Food in Canada.* Market Indicator Report. Accessed February 2017 from: http://www.agr.gc.ca/resources/prod/Internet-Internet/MISB-DGSIM/ATS-SEA/PDF/6245-eng.pdf.

Ajala, O., English, P., Pinkney, J. 2013. *Systematic review and meta-analysis of different dietary approaches to the management of type 2 diabetes.* American Journal of Clinical Nutrition. Vol. 97: 505-519.

Aldrich, G. 2013. *Ingredient Issues.* Petfoodindustry.com/ingredientissues.aspx. Accessed December 2013.

Ambigaipalan, P., Hoover, R., Donner, E., Liu, Q., Jaiswal, S., Chibbar, R., Nantanga, K.K.M., Seetharaman, K. 2011. *Structure of faba bean, black bean and pinto bean starches at different levels of granule organization and their physiochemical properties.* Food Research International. Vol. 44(9): 2962-2974.

Anderson, T.J. 1997. *Oxidative Stress, Endothelial Function, and Coronary Atherosclerosis.* Cardiologia. Vol. 42: 701-714.

Ankrah, N., Appiah-Opong, R. 1999. *Toxicity of Low Levels of Methylglyoxal: Depletion of Blood Glutathione and Adverse Effect on Glucose Tolerance in Mice.* Toxicology Letters. Vol. 109: 61-67.

Appleton, D.J., Rand, J.S., Priest, J., Sunvold, G.D., Vickers, J.R. 2004. *Dietary carbohydrate source affects glucose concentrations, insulin secretion, and food intake in overweight cats.* Nutrition Research. Vol. 24: 447-467.

Arendt, M., Fall, T., Lindblad-Toh, K., Axelsson, E. 2014. *Amylase activity is associated with AMY2B copy numbers in dog: implications for dog domestication, diet and diabetes.* Animal Genetics. Vol. 45:716–722.

Arikawa, A.Y., Jakits, H.E., Flood, A., Thomas, W., Gross, M., Schmitz, K.H., Kurzer, M.S. 2015. *Consumption of a high glycemic load but not a high glycemic index diet is marginally associated with oxidative stress in young women.* Nutrition Research. Vol. 35(1): 7-13.

Association of American Feed Control Officials. 2009. *Official Publication: Association of American Feed Control Officials Incorporated*. Oxford, IN: Association of American Feed Control Officials.

Association of American Feed Control Officials. 2014. *Official Publication: Association of American Feed Control Officials Incorporated*. Oxford, IN: Association of American Feed Control Officials.

Backus, R.C., Cave, N.J., Keisler, D.H. 2007. *Gonadectomy and high dietary fat but not high dietary carbohydrate induce gains in body weight and fat of domestic cats.* British Journal of Nutrition. Vol. 98: 641-650.

Bao, J., Atkinson, F., Petocz, P., Willett, W.C., Brand-Miller, J.C. 2011. *Prediction of postprandial glycemia and insulinemia in lean, young, healthy adults: glycemic load compared with carbohydrate content alone.* American Journal of Clinical Nutrition. Vol. 93: 984-996.

Barclay, A.W., Petocz, P., McMillan-Price, J., Flood, V.M., Prvan, T., Mitchell, P., Brand-Miller, J.C. 2008. *Glycemic index, glycemic load, and chronic disease- a meta-analysis of observational studies.* The American Journal of Clinical Nutrition. Vol. 87(3): 627-637.

Batchelor, D.J., Al-Rammahi, M., Moran, A.W., Brand, J.G., Li, X., Haskins, M., German, A.J., Shirazi-Beechey, S.P. 2011. *Sodium/Glucose Cotransporter-1, Sweet Receptor, and Disaccharidase Expression in the Intestine of the Domestic Dog and Cat: Two Species of Different Dietary Habit*. Am J Physiol Regul Integr Comp Physiol. Vol. 300: R67-R75.

Bazolli, R.S., Vasconcellos, R.S., de-Oliveira, L.D., Sa, F.C., Pereira, G.T., Carciofi, A.C. 2015. *Effect of the particle size of maize, rice, and sorghum in extruded diets for dogs on starch gelatinization, digestibility, and the fecal concentration of fermentation products*. Journal of Animal Science. Vol. 93(6): 2956-2966.

Bernabé, A.M., Srikaeo, K., Schluter, M. 2011. *Resistant starch content, starch digestibility and the fermentation of some tropical starches in vitro*. Food Digestion. Vol. 2(1): 37-42.

Berrios, J.De.J., Morales, P., Camara, M., Sanchez-Mata, M.C. 2010. *Carbohydrate composition of raw and extruded pulse flours.* Food Research International. Vol. 43(2): 531-536.

Björck, I., Gunnarsson, A., Östergård, K. 1989. *A study of native and chemically modified potato starch. Part II: Digestibility in the rat intestinal tract*. Starch. Vol. 41: S128–S134.

Bland, I.M., Guthrie-Jones, A., Taylor, R.D., Hill, J. 2010. *Dog Obesity: Veterinary Practices' and Owners' Opinions on Cause and Management.* Preventative Veterinary Medicine. Vol. 94: 310-315.

Boes, U., Klaus, E., Dittrich, P., Wascher, T.C. 1999. *Postprandial increases of circulating methylglyoxal in type-II diabetic and non-diabetic subjects.* Abstract presented for: 35th Annual Meeting of the European Association for the study of Diabetes. Diabetologia. Vol 42: A327.

Bosch, G., Hagen-Plantinga, E.A., Hendriks, W.H. 2015. *Dietary nutrient profiles of wild wolves: insights for optimal dog nutrition?* British Journal of Nutrition. Vol. 113(S1): S40-S54.

Bouchard, G.F., Sunvold, G.D. 1999. *Improving canine glycemic response to a meal with dietary starch.* Proceedings of the North American Veterinary Conference: 16-19.

Bouchard, G.F., Sunvold, G.D. 2000. *Effect of dietary carbohydrate source on postprandial plasma glucose and insulin concentrations in cats.* In Recent Advances in Canine and Feline Nutrition, Iams Nutrition Symposium Proceedings. Vol.3: 91-101. Orange Frazer Press, Wilmington, OH, USA.

Boye, J., Zare, F., Pletch, A. 2010. *Pulse proteins: processing, characterization, functional properties and applications in food and feed.* Food Research International. Vol. 43: 414-431.

Brand-Miller, J., McMillan-Price, J., Steinbeck, K., Caterson, I. 2009. *Dietary glycemic index: health implications.* Journal of the American College of Nutrition. Vol. 28(4): 446S-449S.

Brosey, B.P., Hill, R.C., Scott, K.C. 2000. *Gastrointestinal volatile fatty acid concentrations and pH in cats.* American Journal of Veterinary Research. Vol. 61(4): 359-361.

Buddington, R.K., Chen, J.W., Diamond, J.M. 1991. *Dietary regulation of intestinal brush-border sugar and amino acid transport in carnivores.* American Journal of Physiology. Vol. 261(4): R793-801.

Canadian Diabetes Association. 2013. http://www.diabetes.ca. Retrieved November 2013.

Canadian Veterinary Medical Association. 2011. *Canada's Pet Wellness Report.* Accessed February 2017 from: https://www.canadianveterinarians.net/documents/canada-s-pet-wellness-report2011.

Carciofi, A.C., Takakura, F.S., de-Oliveira, L.D., Teshima, E., Jeremias, J.T., Brunetto, M.A., Prada, F. 2008. *Effects of Six Carbohydrate Sources on Dog Diet Digestibility and Postprandial Glucose and Insulin Response*. Journal of Animal Physiology and Animal Nutrition. Vol. 92: 326-336.

Case, L.P., Hayek, M.G., Daristotle, L., Raasch, M.F. 2011. *Canine and Feline Nutrition: A Resource for Companion Animal Professionals*. Mosby, Inc. Missouri, USA.

Ceriello, A. 2002. *Nitrotyrosine: New Findings as a Marker of Postprandial Oxidative Stress.* International Journal of Clinical Practice. Vol. 129: 51-58.

Ceriello, A., Esposito, K., Piconi, L., Ihnat, M.A., Thorpe, J.E., Testa, R., Boemi, M., Giugliano, D. 2008. *Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients.* Diabetes. Vol. 57: 1349-1354.

Champ, M. 2002. *Non-nutrient bioactive substances of pulses.* British Journal of Nutrition. Vol. 88(3): S307-S319.

Champ, M., Langkilde, A., Brouns, F., Kettlitz, B., Collet, Y. 2003. *Advances in dietary fibre characterisation. 1. Definition of dietary fibre, physiological relevance, health benefits and analytical aspects*. Nutrition Research Reviews. Vol. 16: 71-82.

Chandler, M., Cunningham, S., Lund, E.M., Khanna, C., Naramore, R., Patel, A., Day, M.J. 2017. *Obesity and associated comorbidities in people and companion animals: A one health perspective*. Journal of Comparative Pathology. Vol. 156: 296-309.

Chansaisakorn, W., Sriphavatsarakorn, P., Sopakdittapong, P., Trisiriroj, M., Pondeenana, S., Buranakarl, C. 2009. *Oxidative stress and intraerythrocytic concentrations of sodium and potassium in diabetic dogs.* Veterinary Research Communications. Vol. 33(1): 67-75.

Chung, H.J., Liu, Q., Hoover, R., Warkentin, T.D., Vandenberg, B. 2008a. *In vitro starch digestibility, expected glycemic index, and thermal and pasting properties of flours from pea, lentil and chickpea cultivars.* Food Chemistry. Vol.111(2): 316-321.

Chung, H.J., Shin, D.H., Lim, S.T. 2008b. *In vitro starch digestibility and estimated glycemic index of chemically modified cornstarches*. Food Research International. Vol. 41: 579-585.

Chung, H.J., Liu, Q. 2012. *Physiochemical properties and in vitro digestibility of flour and starch from pea (Pisum sativum L.) cultivars.* International Journal of Biological Macromolecules. Vol 50: 131-137.

Church, D.B. 1980. *A comparison of intravenous and oral glucose tolerance tests in the dog.* Research in Veterinary Science. Vol. 29(3): 353-359.

Coradini, M., Rand, J.S., Morton, J.M., Filippich, L.J. 2006. *Delayed gastric emptying may contribute to prolonged postprandial hyperglycemia in meal-fed cats*. Journal of Veterinary Internal Medicine. Vol. 20: 726–727.

Courcier, E.A., Thomson, R.M., Mellor, D.J., Yam, P.S. 2010. *An Epidemiological Study of Environmental Factors Associated with Canine Obesity*. Journal of Small Animal Practice. Vol. 51: 362-367.

Cummings, J.H., Englyst, H.N. 1995. *Gastrointestinal effects of food carbohydrate.* Americano Journal of Clinical Nutrition. Vol. 61(4): 938-945.

Del Guerra, S., Lupi, R., Marselli, L., Masini, M., Bugliani, M., Sbrana, S., Torri, S., Pollera, M., Boggi, U., Mosca, F., Del Prato, S., Marchetti, P. 2005. *Functional and Molecular Defects of Pancreatic Islets in Human Type 2 Diabetes*. Diabetes. Vol. 54: 727-735.

De-Oliveira, L.D., Carciofi, A.C., Oliveira, M.C.C., Vasconcellos, R.S., Bazolli, R.S., Pereira, G.T., Prada, F. 2008. *Effects of six carbohydrate sources on diet digestibility and postprandial glucose and insulin responses in cats*. Journal of Animal Science. Vol. 86: 2237-2246.

Deng, P., Swanson, K.S. 2014. *Companion animal symposium: future aspects and perceptions of companion animal nutrition and sustainability.* Journal of Animal Science. Vol. 93(3): 823-834.

Desai, K. M., Chang, T., Wang, H., Banigesh, A., Dhar, A., Liu, J., Untereiner, A., Wu, L. 2010. *Oxidative stress and aging: is methylglyoxal the hidden enemy?* Canadian Journal of Physiology and Pharmacology. Vol.88(3): 273-284.

Dhar, A., Desai, K., Kazachmov, M., Yu, P., Wu, L. 2008. *Methylglyoxal Production in Vascular Smooth Muscle Cells from Different Metabolic Precursors*. Metabolism Clinical and Experimental. Vol. 57: 1211-1220.

Dhar, A., Dhar, I., Desai, K.M., Wu, L. 2010. *Methylglyoxal scavengers attenuate endothelial dysfunction induced by methylglyoxal and high concentrations of glucose.* British Journal of Pharmacology. Vol. 161(8): 1843-1856.

Dhar, A., Dhar, I., Jiang, B., Desai, K.M, Wu, L. 2011. *Chronic Methylglyoxal Infusion by Minipump causes Pancreatic [beta]-Cell Dysfunction and Induces Type 2 Diabetes in Sprague-Dawley Rats.* Diabetes. Vol. 60: 899-908.

Dhar, I., Dhar, A., Wu, L., Desai, K.M. 2013. Increased Methylglyoxal Formation with Upregulation of Renin Angiotensin System in Fructose Fed Sprague Dawley Rats. *PLoS One.* Vol. 8(9): e74212.

Dhar, I., Dhar, A., Lingyun, W., Desai, K. M. 2014. *Methylglyoxal, a reactive glucose metabolite, increases renin angiotensin aldosterone and blood pressure in male Sprague-dawley rats.* American Journal of Hypertension. Vol. 27(3): 308-316.

Di Carli, M.F., Janisse, J., Grunberger, G., Ager, J. 2003. *Role of Chronic Hyperglycemia in the pathogenesis of Coronary Microvascular Dysfunction in Diabetes*. Journal of the American College of Cardiology. Vol. 41: 1387-1393.

Dickinson, S., Hancock, D.P., Petocz, P., Ceriello, A., Brand-Miller, J. 2008. *High-glycemic index carbohydrate increases nuclear factor-κB activation in mononuclear cells of young, lean healthy subjects.* American Journal of Clinical Nutrition. Vol. 87(5): 1188-1193.

Eerlingen, R.C., Jacobs, H., Delcour, J.A. 1994. Enzyme Resistant Starch. V. *Effect of Retrogradation of Waxy Maize Starch on Enzyme Susceptibility*. Cereal Chemistry. Vol. 71: 351-355.

Egner, B., Carr, A., Brown, S. 2003. *Essential Facts of Blood Pressure in Dogs and Cats*. Beate Egner Vet Verlag. Germany.

Eisert, R. 2011. *Hypercarnivory and the brain: protein requirements of cats reconsidered*. Journal of Comparative Physiology. Vol. 181(1): 1-17.

Elliott, K.F., Rand, J.S., Fleeman, L.M., Morton, J.M., Litster, A.L., Biourge, V.C., Markwell, P.J. 2012. *A diet lower in digestible carbohydrate results in lower postprandial glucose concentrations compared with a traditional canine diabetes diet and an adult maintenance diet in healthy dogs.* Research in Veterinary Science. Vol. 93: 288-295.

Englyst, H.N., Kingman, S.M., Cummings, J.H. 1992. *Classification and measurement of nutritionally important starch fractions.* European Journal of Clinical Nutrition. Vol. 46: S33-S50.

Erayu, R., Shrestha, A.K., Arcot, J. 2009. *Effect of Various Processing Techniques on Digestibility of Starch in Red Kidney Bean (Phaseolus vulgaris) and Two Varieties of Peas (Pisum sativum).* Food Research International. Vol. 42: 956-962.

Falkenö, U., Hillström, A., von Brömssen, C., Strage, E.M. 2016. *Biological variation of 20 analytes measured in serum from clinically healthy domestic cats.* Journal of Veterinary Diagnostic Investigations. Vol. 28(6): 699-704.

Farrow, H.A., Rand, J.S., Sunvold, G.D. 2002. *The effect of high protein, high fat or high carbohydrate diets on postprandial glucose and insulin concentrations in normal cats*. Journal of Veterinary Internal Medicine. Vol. 16: 360.

Farrow, H.A., Rand, J.S., Morton, J.M., Sunvold, G.D. 2012. *Postprandial glycaemia in cats fed a moderate carbohydrate meal persists for a median of 12 hours- female cats have higher peak glucose concentrations*. Journal of Feline Medicine and Surgery. Vol. 14(10): 706-715.

Farrow, H.A., Rand, J.S., Morton, J.M., O'Leary, C.A., Sunvold, G.D. 2013. *Effect of dietary carbohydrate, fat and protein on postprandial glycemia and energy intake in cats.* Journal of Veterinary Internal Medicine. Vol. 27: 1121-1135.

Fischer, M.M., Kessler, A.M., de Sa, L., Vasconcellos, R.S., Roberti Filho, F.O., Nogueira, S.P., Oliveira, M.C., Carciofi, A.C. 2012. *Fibre fermentability effects on energy and macronutrient digestibility, fecal traits, postprandial metabolite responses, and colon histology of overweight cats.* Journal of Animal Science. Vol. 90(7): 2233-2245.

Fiory, F., Lombardi, A., Miele, C., Guidicelli, J., Beguinot, F., Obberghen, E. 2011. *Methylglyoxal Impairs Insulin Signalling and Insulin Action on Glucose-Induced Insulin Secretion in the Pancreatic Beta Cell Line INS-1E*. Diabetologia. Vol. 55: 2941-2952.

Fleischer, S., Sharkey, M., Mealey, K., Ostrander, E.A., Martinez, M. 2008. *Pharmacogenetic and metabolic differences between dog breeds: their impact on canine medicine and the use of the dog as a preclinical animal model.* The American Association of Pharmaceutical Sciences Journal. Vol. 10(1): 110-119.

Fleming, R.M., Boyd, L.B. 2000. *The effect of high protein diets on coronary blood flow.* Angiology. Vol. 51: 817-826.

Food and Agriculture Organization of the United Nations (FAO). 2003. *Food energy- methods of analysis and conversion factors.* FAO food and nutrition paper 77: Report of a technical workshop, Rome. Accessed February 2017 from: http://fao.org.

Food and Agriculture Organization of the United Nations (FAO). *Health benefits of pulses.* Accessed from: fao.org, May 2017.

Foster-Powell, K., Miller, J.B. 1995. *International Tables of Glycemic Index.* American Journal of Clinical Nutrition. Vol. 62(4): 871S-893S.

Foster-Powell, K., Holt, S.H.A, Brand-Miller, J.C. 2002. *International Table of Glycemic Index and Glycemic Load Values.* American Journal of Clinical Nutrition. Vol 76-1: 5-56.

Frank, G., Anderson, W., Pazak, H., Hodgkins, E., Ballam, J., Laflamme, D. 2001. *Use of a high-protein diet in the management of feline diabetes mellitus*. Vet. Ther. Vol. 2: 238–246.

Genovese, M.I., Lajolo, F.M. 2001. *Trypsin inhibitory activity of beans (Phaseolus vulgaris L.): critical evaluation of methods for determination.* Archivos Latinoamericanos de Nutricion. Vol. 51(4): 386–394.

German, A.J., Hervera, M., Hunter, L., Holden, S.L., Morris, P.J., Biourge, V., Trayhurn, P. 2009. *Improvement in Insulin Resistance and Reduction in Plasma Inflammatory Adipokines After Weight Loss in Obese Dogs*. Domestic Animal Endocrinology. Vol. 37: 214-226.

German, A.J., Ryan, V.H., German, A.C., Trayhurn, P., Wood, I.S. 2010. *Obesity, its Associated Disorders and the Role of Inflammatory Adipokines in Companion Animals*. The Veterinary Journal. Vol. 185: 4-9.

Goni, I., Garcia-Alonso, A., Saura-Calixto, F. 1997. *A starch hydrolysis procedure to estimate glycemic index.* Nutrition Research. Vol. 17: 427-437.

Gunawardena, C.K., Zijlstra, R.T., Goonewardene, L.A., Beltranena, E. 2010. *Protein and starch concentrates of air classified field pea and zero-tannin faba bean for weaned pigs.* Journal of Animal Science. Vol. 88: 2627-2636.

Guyton, A.C., Hall, J.E. 1997. *Human Physiology and Mechanisms of Disease*. Sixth Edition. W.B. Saunders Company. Philadelphia, PA. Ch.13. Section 52: 625-633.

Han, Y., Randell, E., Vasdev, S., Gill, V., Gadag, V., Newhook, L.A., Grand, M., Hagerty, D. 2007. *Plasma methylglyoxal and glyoxal are elevated and related to early membrane alteration in young, complication-free patients with type 1 diabetes.* Molecular Cell Biochemistry. Vol. 305: 123-131.

Harris, W.S., Connor, W.E., Alam, N., Illingworth, D.R. 1988. *Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids*. Journal of Lipid Research. Vol. 29: 1451-1460.

Hediger, M.A., Rhoads, D.B. 1994. *Molecular Physiology of Sodium-Glucose Cotransporters*. Physiological Reviews. Vol. 74: 993-1026.

Henry, C.J., Lightowler, H.J., Newens, K.J., Pata, N. 2008. *The Influence of Adding Fats of Varying Saturation on the Glycemic Response of White Bread*. International Food Journal of Food Science and Nutrition. Vol. 59: 61-69

Hewson-Hughes, A.K., Gilham, M.S., Upton, S., Colyer, A., Butterwick, R., Miller, A.T. 2011. *The Effect of Dietary Starch Level on Postprandial Glucose and Insulin Concentrations in Cats and Dogs*. British Journal of Nutrition. Vol. 106: S105-S109.

Hipkiss, A.R. 2005. *Glycation, ageing and carnosine: Are carnivorous diets beneficial?* Mechanisms of ageing and development. Vol. 126(10): 1034-1039.

Hoenig, M., Thomaseth, K., Waldron, M., Ferguson, D.C. 2007. *Insulin sensitivity, fat distribution, and adipocytokine response to different diets in lean and obese cats before and after weight loss.* American Journal of Physiology. Vol. 292: 227-234.

Hoenig, M., Jordan, E.T., Ferguson, D.C., de-Vries, F. 2010. *Oral Glucose Leads to a Differential Response in Glucose, Insulin, and GLP-1 in Lean vs Obese Cats*. Domestic Animal Endocrinology. Vol. 38: 95-102.

Hoenig, M., Jordan, E.T., Glushka, J., Kley, S., Patil, A., Waldron, M., Prestegard, J.H., Ferguson, D.C., Wu, S., Olson, D.E. 2011. *Effects of macronutrients, age and obesity on 6 and 24-hour postprandial glucose metabolism in cats.* American Journal of Physiology. Vol. 301(6): R1798-R1807.

Hoenig, M. 2012. *The cat as a model for human obesity and diabetes.* Journal of Diabetes Science and Technology. Vol. 6: 525-533.

Hoover, R., Hannouz, D., Sosulski, F.W. 1988. *Effects of hydroxypropylation on thermal properties, starch digestibility and freeze-thaw stability of field pea (Pisum sativum cv Trapper).* Starch. Vol. 40: 383-387.

Hoover, R., Hughes, T., Chung, H.J. Liu, Q. 2010. *Composition, Molecular Structure, Properties and Modifications of Pulse Starches: A Review*. Food Research International. Vol. 43: 399-413.

Howarth, N.C., Saltzman, E., Roberts, S.B. 2001. *Dietary fibre and weight regulation.* Nutrition Review. Vol. 59: 129-139.

Ignarro, L.J., Buga, G.M., Wood, K.S., Byrnes, R.E., Chaudhuri, G. 1987. *Endothelium Derived Relaxing Factor Produced and Released from Artery and Vein is Nitric Oxide*. Proceedings of the National Academy of Sciences of the United States of America. Vol. 84: 9265-9269.

Jayalath, V.H., de Souza, R.J., Sievenpiper, J.L., Ha, V., Chiavaroli, L., Mirrahimi, A., Di Buono, M., Bernstein, A.M., Leiter, L.A., Kris-Etherton, P.M., Vuksan, V., Beyene, J., Kendall, C.W., Jenkins, D.J. 2014. *Effect of dietary pulses on blood pressure: a systematic review and meta-analysis of controlled feeding trials.* American Journal of Hypertension. Vol. 27(1): 56-64.

Jenkins, D.J., Wolever, T.M., Taylor, R.H., Barker, H., Fielden, H., Baldwin, J.M., Bowling, A.C., Newman, H.C., Jenkins, A.L., Goff, D.V. 1981. *Glycemic Index of Foods: A Physiological*

Basis for Carbohydrate Exchange. The American Journal of Clinical Nutrition. Vol. 34: 362-366.

Jenkins, D.J., Kendall, C.W., Augustin, L.S., Franceschi, S., Hamidi, M., Marchie, A., Jenkins, A.L., Axelsen, M. 2002. *Glycemic index: overview of implications in health and disease.* The American Journal of Clinical Nutrition. Vol. 76(1): 266S-273S.

Jia, X., Olson, D.J.H., Ross, A.R.S., Wu, L. 2006. *Structural and Functional Changes in Human Insulin Induced by Methylglyoxal*. FASEB Journal. Vol. 20: E871-E879.

Jones, I.D., Luis Fuentes, V., Fray T.R., Vallance, C., Elliott, J. 2010. *Evaluation of a flow-mediated vasodilation measurement technique in healthy dogs.* American Journal of Veterinary Research. Vol. 71: 1154-1161.

Kalapos, M.P. 1999. *Methylglyoxal in Living Organisms: Chemistry, Biochemistry, Toxicology and Biological Implications*. Toxicology Letters. Vol. 110: 145-175.

Kalapos, M.P. 2013. *Where does plasma methylglyoxal originate from?* Diabetes Research and Clinical Practice. Vol. 99(3): 260-271.

Kawano, H., Motoyama, T., Hirashima, O., Hirai, N., Miyao, Y., Sakamoto, T., Kugiyama, K., Ogawa, H., Yasue, H. 1999. *Hyperglycemia Rapidly Suppresses Flow-mediated Endothelial-dependant Vasodilation of Brachial Artery*. Journal of the American College of Cardiology. Vol. 34: 136-154.

Kienzle, E. 1993a. *Carbohydrate metabolism of the cat. 1. Activity of amylase in the gastrointestinal tract of the cat.* Journal of Animal Physiology and Animal Nutrition. Vol. 69: 92-101.

Kienzle, E. 1993b. *Carbohydrate metabolism of the cat. 2. Digestion of starch.* Journal of Animal Physiology and Animal Nutrition. Vol. 69: 102-114.

Kienzle, E. 1994a. *Blood sugar levels and renal sugar excretion after the intake of high carbohydrate diets in cats.* Journal of Nutrition. Vol 124(12): 2563S-2567S.

Kienzle, E. 1994b. *Effect of carbohydrates on digestion in the cat.* Journal of Nutrition. Vol. 124: 2568S-2571S.

Kim, S.J., de Souza, R.L., Choo, V.L., Ha, V., Cozma, A.I., Chiavaroli, L., Mirrahimi, A., Blanco Mejia, S., Di Buono, M., Bernstein, A.M., Leiter, L.A., Kris-Etherton, P.M., Vuksan, V., Beyene, J., Kendall, C.W., Jenkins, D.J., Sievenpiper, J.L. 2016. *Effects of dietary pulse consumption on body weight: a systematic review and meta-analysis of randomized controlled trials*. American Journal of Clinical Nutrition. Vol. 103: 1213–1223.

Kitamura, Y., Yasuda, J., Hashimoto, A. 1999. *Acute insulin response to intravenous arginine in nonobese healthy cats.* Journal of Veterinary Internal Medicine. Vol. 13: 549-556.

Koeppen, B. & Stanton, B. 2017. *Bern & Levy Physiology: 7th edition*. Philadelphia, PA. USA. Elsevier.

Kojda, G., Harrison, D. 1999. *Interaction between NO and Reactive Oxygen Species: Pathophysiological Importance in Atherosclerosis, Hypertension, Diabetes, and Heart Failure*. Cardiovascular Research. Vol. 43: 562-571.

Korus, J., Witczak, M., Juszczak, L, Ziobro, R. 2008. *Grass pea (*Lathyrus sativus *L.) starch as an alternative for cereal starches: Rheological properties and retrogradation susceptibility*. Journal of Food Engineering. Vol. 88(4): 528-534.

Krajcovicova-Kudlackova, M., Sebekova, K., Schinzel, R., Klvanova, J. 2002. *Advanced glycation end products and nutrition.* Physiological Research. Vol. 51(3): 313-316.

Laflamme, D. 1997a. *Development and validation of a body condition score system for dogs.* Canine Practice. Vol. 22: 10-15.

Laflamme, D.P. 1997b. *Development and validation of a body condition score system for cats: A clinical tool.* Feline Practice. Vol. 25: 13-18.

Laflamme, D., Abood, S.K., Fascetti, A.J., Fleeman, L.M., Freeman, L.M., Michel, K.E., Bauer, C., Kemp, B. L. E., Van Doren, J.R., Willoughby, K.N. 2008. *Pet feeding practices of dog and cat owners in the United states and Australia.* Journal of the American VeterinaryMedical Association. Vol. 232(5): 687-694.

Laflamme, D., Izquierdo, O., Eirmann, L.A., Binder, S. 2014. *Myths and misperceptions about ingredients used in commercial pet foods.* The veterinary clinics of North America: Small animal practice. Vol. 44(4): 689-698.

Larson, B.T., Lawler, D.F., Spitznagel, E.L., Kealy, R.d. 2003. *Improved Glucose Tolerance with Lifetime Diet Restriction Favorably Affects Disease and Survival in Dogs*. Journal of Nutrition. Vol. 133: 2887-2892.

Legrand-Defretin, V. 1994. *Differences Between Cats and Dogs: A Nutritional Review*. Proceedings of the Nutrition Society. Vol. 53: 15-24.

Lehmann, U., Robin, F. 2007. *Slowly Digestible Starch- Its Structure and Health Implications.* Trends in Food Science and Technology. Vol. 18: 346-355.

Leray, V., Siliart, B., Dumon, H., Martin, L., Serqheraert, R., Biourge, V., Nguyen, P. 2006. *Protein intake does not affect insulin sensitivity in normal weight cat.* Journal of Nutrtion. Vol. 136(7): 2028S-2030S.

Loader, J., Montero, D., Lorenzen, C, Watts, R., Meziat, C., Reboul, C., Stewart, S., Walther, G. 2015. *Acute hyperglycemia impairs vascular function in healthy and cardiometabolic diseased subjects: systematic review and meta-analysis.* Arteriosclerosis, Thrombosis and Vascular Biology. Vol. 35: 2060-2072.

Ludwig, D.S. 2002. *The glycemic index: physiological mechanisms relating to obesity, diabetes, and cardiovascular disease.* Journal of the American Medical Association. Vol. 287(18): 2414-2423.

Lund, E., Armstrong, P.J., Kirk, C.A., Klausner, J.S. 2005. *Prevalence and risk factors for obesity in adult cats from private US veterinary practices.* International Journal of Applied Research in Veterinary Medicine. Vol. 3: 88-96.

Lund, E., Armstrong, P.J., Kirk, C.A., Klausner, J.S. 2006. *Prevalence and risk factors for obesity in adult dogs from private US veterinary practices*. International Journal of Applied Research in Veterinary Medicine.Vol 4: 177-186.

MacDonald, M.L., Rogers, Q.R., Morris, J.G. 1984. *Nutrition of the domestic cat, a mammalian carnivore.* Annual Review of Nutrition. Vol 4: 521-562.

MacLean, H. 1924. *Insulin*. Nature. Vol. 114: 33-40.

Marinangeli, C.P.F., Jones, P.J.H. 2011. *Whole and fractionated yellow pea flours reduce fasting insulin and insulin resistance in hypercholesterolaemic and overweight human subjects.* British Journal of Nutrition. Vol. 105: 110-117.

Marti, C.N., Gheorghiade, M., Kalogeropoulos, A.P., Georgiopoulou, V.V., Quyyumi, A.A., Butler, J. 2012. *Endothelial Dysfunction, Arterial Stiffness, and Heart Failure*. Journal of the American College of Cardiology. Vol. 60: 1455-1469.

Martin, L.J.M., Lutz, T.A., Daumas, C., Bleis, P., Nguyen, P., Biourge, V., Dumon, H.J.W. 2014. *Acute hormonal response to glucose, lipids and arginine infusion in overweight cats.* Journal of Nutritional Science. Vol. 3: e8, 1-10.

Masania, J., Malczewska-Malec, M., Razny, U., Goralska, J., Zdzienicka, A., Kiec-Wilk, B., Gruca, A., Stancel-Mozwillo, J., Dembinska-Kiec, A., Rabbani, N., Thornalley, P.J. 2016. *Dicarbonyl stress in clinical obesity.* Glycoconjugate Journal. Vol. 33(4): 581-589.

Maskell, I.E., Johnson, J.V. 1993. *Digestion and absorption*. In: The Waltham Book of Companion Animal Nutrition: I. H. Burger (Ed.). p 25. Pergamon Press, New York.

Masterjohn, C., Mah, E., Guo, Y., Koo, S, I., Bruno, R.S. 2012. *Γ-Tocopherol abolishes postprandial increases in plasma methylglyoxal following an oral dose of glucose in healthy, college aged men.* The Journal of Nutritional Biochemistry. Vol 23(3): 292-298.

Matafome, P., Santos-Silva, D., Crisostomo, J., Rodrigues, T., Rodrigues, L., Sena, C.M., Pereira, P., Seica, R. 2012. *Methylglyoxal causes structural and functional alterations in adipose tissue independently of obesity*. Archives of Physiology and Biochemistry. Vol. 118: 58-68.

Matsukawa, K. & Ninomiya, I. 1987. *Changes in renal sympathetic nerve activity, heart rate and arterial blood pressure associated with eating in cats.* Journal of Physiology. Vol. 390: 229-242.

McLellan, A.C., Thornalley. P.J., Benn, J., Sonksen, P.H. 1994. *Glyoxalase system in clinical diabetes mellitus and correlation with diabetic complications.* Clinical Science. Vol. 87: 21-29.

Merck Manual. Veterinary Manual. *Serum biochemical reference ranges.* Accessed August 2017 from: http://www.merckvetmanual.com/appendixes/reference-guides/serum-biochemical-reference-ranges.

Mey, C.D., Enterling, D., Meineke, I. 1993. *Cardiovascular effects of eating, atenolol and their interaction: β_1-adrenergic modulation does not play a predominant role in the genesis of postprandial effects.* British Journal of Clinical Pharmacology. Vol. 36(5): 427-435.

Meyer, H., Kienzle, E. 1991. *Dietary protein and carbohydrates: relationship to clinical disease.* Proceedings of the Purina International Nutrition Symposium. 13-26.

Miyazaki, H., Yoshida, M., Samura, K., Matsumoto, H., Ikemoto, F., Tagawa, M. 2002. *Ranges of diurnal variation and the pattern of body temperature, blood pressure and heart rate in laboratory beagle dogs.* Experimental Animals. Vol. 51(1): 95-98.

Mokdad, A.H., Ford, E.S., Bowman, B.A., Dietz, W.H., Vinicor, F., Bales, V.S., Marks, J.S. 2003. *Prevelance of Obesity, Diabetes, and Obesity-Related Health Risk Factors*. The Journal of the American Medical Association. Vol. 289: 76-79.

Mori, A., Ueda, K., Lee, P., Oda, H., Ishioka, K., Sako, T. 2016. *Influence of various carbohydrate sources on postprandial glucose, insulin and NEFA concentrations in obese cats.* Polish Journal of Veterinary Sciences. Vol. 19(2): 387-391.

Mudryj, A.N., Yu, N., Aukema, H.M. 2014. *Nutritional and health benefits of pulses.* Applied Physiology, Nutrition and Metabolism. Vol. 39(11): 1197-1204.

Mukohda, M., Morita, T., Okada, M., Hara, Y., Yamawaki, H. 2013. *Long-term methylglyoxal treatment causes endothelial dysfunction of rat isolated mesenteric artery.* Journal of Veterinary Medical Science. Vol. 75(2): 151-157.

Murray, S.M., Fahey, G.C., Merchen, N.R., Sunvold, G.D., Reinhart, G.A. 1999. *Evaluation of selected high-starch flours as ingredients in canine diets.* Journal of Animal Science, Vol. 77: 2180-2186.

National Research Council. 2006. *Nutrient requirements of dogs and cats.* The National Academic Press. Washington, D.C.

Nelson, R.W & Reusch, C.E. 2014. *Animal models of disease: Classification and etiology of diabetes in dogs and cats.* Journal of Endocrinology. Vol. 222: T1-T9.

Nemec, A., Verstaete, F.J.M., Jerin, A., Sentjurc, M., Kass, P.H., Petelin, M., Pavlica, Z. 2013. *Peridontal disease, periodontal treatment and systemic nitric oxide in dogs.* Research in Veterinary Science. Vol. 94(3): 542-544.

Nguyen, P., Dumon, H., Biourge, V., Pouteau, E. 1998. *Glycemic and insulinemic responses after ingestion of commercial foods in healthy dogs: influence of food composition.* The Journal of Nutrition. Vol.128: 2654S-2658S.

Nunomura, A., Perry, G., Aliev G., Hirai, K., Takeda, A., Balraj, E.K., Jones, P.K., Ghanbari, H., Wataya, T., Shimohama, S., Chiba, S., Atwood, C.S., Petersen, R.B., Smith, M.A. 2001. *Oxidative Damage is the Earliest Event in Alzheimer Disease*. Journal of Neuropathology and Experimental Neurology. Vol. 60: 759-767.

Nyland, T.G., Mattoon, J.S. 2002. *Small Animal Diagnostic Ultrasound*. W.B. Saunders Company. United States of America.

Olefsky, J.M., Farquhar, J.W., Reaven, G.M. 1973. *Do the oral and intravenous glucose tolerance test provide similar diagnostic information in patients with chemical diabetes mellitus?* Diabetes. Vol. 22(3): 202-209.

Pagan, J.D. 2011. *Fermentation key for wide range of species.* Vol. 83(5): 10.

Parada, J., Aguilera, J.M. 2011. *Starch Matrices and the Glycemic Response*. Food Science and Technology International. Vol. 17: 187-205.

Peacock, J., Chur-Hansen, A., Winefield, H. 2012. *Mental health implications of human attachment co companion animals.* Journal of Clinical Psychology. Vol. 68(3): 292-303.

Pereira, P., de Almeida, C.D., Alfenas, R. 2014. *Glycemic index role on visceral obesity, subclinical inflammation and associated chronic diseases.* Nutricion Hospitalaria. Vol 30(2): 237-243.

Pet Food Industry. *9 out of 10 pet owners want pet food ingredient transparency.* Accessed online February 2017 from: http://www.petfoodindustry.com.

Pet Food Industry. *Infographic: popular legumes in dry, wet pet foods.* Accessed online June 2017 from: http://www.petfoodindustry.com.

Pet Food Industry. *Infographic: the future of plant based pet food proteins.* Accessed online June 2017 from: http://www.petfoodindustry.com.

Piccione, G., Caola, G., Refinetti, R. 2005. *Daily rhythms of blood pressure, heart rate and body temperature in fed and fasted male dogs.* Journal of Veterinary Medicine. Vol. 52(8): 377-381.

Plantinga, E.A., Bosch, G., Hendriks, W.H. 2011. *Estimation of the dietary nutrient profile of free-roaming feral cats: possible implications for nutrition of domestic cats.* British Journal of Nutrition. Vol. 106: S35-S48.

Poulsen, M.W., Andersen, J.M., Hedegaard, R.V., Madsen, A.N., Krath, B.N., Monosik. R., Bak, M.J., Nielsen, J., Holst, B., Skibsted, L.H., Larsen, L.H., Dragsted, L.O. 2016. *Short-term effects of advanced glycation end products in rats.* British Journal of Nutrition. Vol. 115: 629-636.

Prasad, A., Andrews, N.P., Padder, F.A., Husain, M., Quyyumi, A.A. 1999. *Glutathione Reverses Endothelial Dysfunction and Improves Nitric Oxide Bioavailability*. Journal of the American College of Cardiology. Vol. 34: 507-514.

Puglia, G.D., Freeman, L.M., Rush, J.E., King, R.G.P., Crawford, S.L. 2006. *Use of a flow-mediated vasodilation technique to assess endothelial function in dogs.* American Journal of Veterinary Research. Vol. 67(9): 1533-1540.

Rand, J.S., Fleeman, L.M., Farrow, H.A., Appleton, D.J., Lederer, R. 2004. *Canine and feline diabetes mellitus: nature or nurture?* Journal of Nutrition. Vol 134: 2072S-2080S.

Ratnayake, W.S., Hoover, R., Warkentin, T. 2002. *Pea Starch: Composition, Structure and Properties*- A Review. *Starch.* Vol. 54: 217-234.

Reaven, G.M. 2011. *Insulin Resistance: The Link between Obesity and Cardiovascular Disease*. *Medical Clinics of North America*. Vol. 95: 875-892.

Rebello, C.J., Greenway, F.L., Finley, J.W. 2014. *Whole grains and pulses: A comparison of the nutritional and health benefits.* Journal of Agriculture and Food Chemistry. Vol. 62: 7029-7049.

Riccardi, G., Rivellese, A.A., Giacco, R. 2008. *Role of glycemic index and glycemic load in the healthy state, in prediabetes, and in diabetes.* American Journal of Clinical Nutrition. Vol.87(1): 269S-274S.

Rivera-Espinoza, Y. & Gallardo-Navarro, Y. 2010. *Non-dairy probiotic products.* Food microbiology. Vol. 27: 1-11.

Ruaux, C.G., Carney, P.C., Suchodolski, J.S., Steiner, J.M. 2012. *Estimates of biological variation in routinely measured biochemical analytes in clinically healthy dogs.* Veterinary Clinical Pathology. Vol. 41(4): 541-547.

Russo, G., Leopold, J.A., Loscalzo, J. 2002. *Vasoactive Substances: Nitric Oxide and Endothelial Dysfunction in Atherosclerosis.* Vascular Pharmacology. Vol. 38: 259-269.

Sandoe, P., Palmer, C., Corr, S., Astrup, A., Reinhard Bjornvad, C. 2014. *Canine and feline obesity: A one health perspective.* Veterinary Record. Vol. 175(24): 610-616.

Salunkhe, D. 1982. *Legumes in human nutrition: current status and future research needs.* Current Science. Vol. 51: 387-394.

Saskatchewan Pulse Growers. Accessed May 2017 from: www.saskpulse.com.

Sena, C.M., Matafome, P., Crisostomo, J., Rodrigues, L., Fernandes, R., Pereira, P., Seica, R.M. 2012. *Methylglyoxal promotes oxidative stress and endothelial dysfunction.* Pharmacological Research. Vol. 65(5): 497-506.

Shearer, P. 2010. *Literature review: Canine, feline, and human overweight and obesity.* Banfield Pet Hospital, Vancouver, WA, USA. Accessed August 2017 from: https://www.banfield.com/getmedia/ aa3b125b-18a7-4422-8f68-7861db3385b4/e15ce5a8-f5df-4864-9b51- 58383540372b-pdf0.

Shubert, J. 2012. *Dogs and Human Health/Mental Health: From the Pleasure of their Company to the Benefits of their Assistance*. U.S. Army Medical Department Journal. April-June: 21-30.

Singh, J., Dartois, A., Kaur, L. 2010. *Starch Digestibility in Food Matrix: A Review*. Trends in Food Science and Technology. Vol. 21: 168-180.

Slingerland, I. 2008. *Effects of differences in food composition in cats.* Proceedings of the 18th Congress of the European College of Veterinary Internal Medicine- Companion Animals. pp: 155-156.

Slingerland, I., Fazilova, V.V., Plantinga, E.A., Kooistra, H.S., Beynan, A.C. 2009. *Indoor confinement and physical inactivity rather than the proportion of dry food are risk factors in the development of feline type 2 diabetes mellitu.* Veterinary Journal. Vol. 179(2): 247-253.

Sousa Silva, M., Gomes, R.A., Ferreira, A.E., Ponces Freire, A., Cordeiro, C. 2013. *The glyoxalase pathway: the first hundred years... and beyond*. The Biochemical Journal. Vol. 453: 1-15.

Stein, H.H., Lagos, L.V., Casas, G.A. 2016. *Nutritional value of feed ingredients of plant origin fed to pigs.* Animal Feed Science and Technology. Vol. 218: 33-69.

Sun, T., Laerke, H.N., Jorgensen, H., Knudsen, K.E. 2006. *The effect of extrusion cooking of different starch sources on the in vitro and in vivo digestibility in growing pigs.* Animal Feed Science and Technology. Vol 131(1-2): 67-86.

Sunvold, G.D., Fahey, G.C., Merchen, N.R., Bourquin, L.D., Titgemeyer, E.C., Bauer, L.L., Reinhart, G.A. 1995a. *Dietary fibre for cats: in vitro fermentation of selected fibre sources by cat fecal inoculum and in vivo utilization of diets containing selected fibre sources and their blends.* Journal of Animal Science. Vol. 73(8): 2329-2339.

Sunvold, G.D., Hussein, H.S., Fahey, G.C., Merchen, N.R., Reinhart, G.A. 1995b. *In vitro fermentation of cellulose, beet pulp, citrus pulp, and citrus pectin using faecal inoculum from cats, dogs, horses, humans and pigs, and ruminal fluid from cattle.* Journal of Animal Science. Vol. 73: 3639-3648.

Tanaka, A., Inoue, A., Takeguchi, A., Washizu, T., Bonkobara, M., Arai, T. 2005. *Comparison of Expression of Glucokinase Gene and Activites of Enzymes Related to Glucose Metabolism in Livers Between Dog and Cat.* Veterinary Research Communications. Vol. 29: 447-485.

Tate & Lyle Food Starches Brochure. 2016. Accessed from www.tateandlyle.com.

Thiess, S., Becskei, C., Tomsa, K., Lutz, T.A., Wanner, M. 2004. *Effects of high carbohydrate and high fat diet on plasma metabolite levels and on iv glucose tolerance test in intact and neutered male cats.* Journal of Feline Medicine and Surgery. Vol. 6: 207-218.

Thompson, A. 2008. *Ingredients: Where Pet Food Starts*. Topics in Companion Animal Medicine. Vol. 23: 127-132.

Thornalley, P.J. 1996. *Pharmacology of Methylglyoxal: Formation, Modification of Proteins and Nucleic Acids and Enxymatic Detoxification- A Role in Pathogenesis and Antiproliferative Chemotherapy.* Genetic Pharmacology. Vol. 27: 565-573.

Turk, Z., Cavlovic-Naglic, M., Turk, N. 2011. *Relationship of methylglyoxal-adduct biogenesis to LDL and triglyceride levels in diabetics*. Life Science. Vol. 89: 485-490.

Usha-Rani, Y.S., Ramakrishna, M.R., Manjunath, P., Trupti, R.R., Rangaswamy, R. 2013. *A comparative study of pre-prandial and post prandial autonomic nervous system response between obese and non obese young women aged 18-25 years.* International Journal of Pharma and Bio Sciences. Vol 4(3): B239-B258.

Vasdev, S., Ford, C.A., Longerich, L., Gadag, V., Wadhawan, S. 1998. *Role of aldehydes in fructose induced hypertension.* Molecular and Cellular Biochemistry. Vol. 181(1): 1-9.

Verbrugghe, A., Hesta, M., Gommeren, K., Daminet, S., Wuyts, B., Buyse, J., Janssens, G.P.J. 2009. *Oligofructose and inulin modulate glucose and amino acid metabolism through propionate production in normal-weight and obese cats.* British Journal of Nutrition. Vol. 102: 694-702.

Verbrugghe, A., Hesta, M., Van Weyenberg, S., Papadopoulos, G.A., Gommeren, K., Daminet, S., Bosmans, T., Polis, I., Buyse, J., Janssens, G.P.J. 2010. *The glucose and insulin response to isoenergetic reduction of dietary energy sources in a true carnivore: the domestic cat (Felis catus).* British Journal of Nutrition. Vol. 104: 214-221.

Verbrugghe, A., Hesta, M., Daminet, S., Janssens, G.P.J. 2012. *Nutritional Modulation of Insulin Resistance in the True Carnivorous Cat: A Review*. Food Science and Nutrition. Vol. 52: 172-182.

Viviano, K.R., Lavergne, S.N., Goodman, L., VanderWielen, B., Grundahl, L., Padilla, M., Trepanier, L.A. 2009. *Glutathione, cysteine, and ascorbate concentrations in clinically ill dogs and cats.* Journal of Veterinary Internal Medicine. Vol. 23(2): 250-257.

Wang, H., Meng, Q.H., Gordan, J.R., Khandwala, H., Wu, L. 2007. *Proinflammatory and Proapoptotic Effects of Methylglyoxal on Neutrophils from Patients with Type 2 Diabetes Mellitus*. Clinical Biochemistry. Vol. 40: 1232-1239.

Wang, X.L. Rainwater, D.L., Leone, A., Mahaney, M.C. 2004. *Effects of diabetes on plasma nitrotyrosine levels.* Diabetic Medicine. Vol. 21(6): 577-580.

Wang, X., Desai, K., Chang, T., & Wu, L. 2005. *Vascular methylglyoxal metabolism and the development of hypertension.* Journal of Hypertension. Vol. 23(8): 1565-1573.

Wang, X., Jia, X., Chang, T., Desai, K., Wu, L. 2008. *Attentuation of Hypertension Development by Scavenging Methylglyoxal in Fructose-Treated Rats*. Journal of Hypertension. Vol. 26: 765-772.

Wang, S., Copeland, L. 2013. *Molecular Disassembly of Starch Granules During Gelatinization and its Effect on Starch Digestibility: A Review*. Food & Function. Vol. 4: 1564-1580.

Washizu, T., Tanaka, A., Sako, T., Washizu, M, Arai, T. 1999. *Comparison of the activities of enzymes related to glycolysis and gluconeogenesis in the liver of dogs and cats.* Research in Veterinary Science. Vol. 67: 203-204.

Webb, C.B., Falkowski, L. 2009. *Oxidative stress and innate immunity in feline patients with diabetes mellitus: the role of nutrition.* Journal of Feline Medicine and Surgery. Vol. 11(4): 271-276.

Wolever, T.M.S., Jenkins, D.J., Jenkins, A.L., Josse, R.G. 1991. *The glycemic index: methodology and clinical implications.* American Journal for Clinical Nutrition. Vol. 54: 846-854.

Wolever, T.M.S., Bolognesi, C. 1996. *Prediction of glucose and insulin responses of normal subjects after consuming mixed meals varying in energy, protein, fat, carbohydrate and glycemic index.* The Journal of Nutrition. Vol. 126: 2807-2812.

Wolf, W.B., Bauer, L.L., Fahey, G.C. 1999. *Effects of chemical modification in vitro rate and extent of food starch digestion: an attempt to discover a slowly digested starch.* Journal of Food and Agriculture Chemistry. Vol. 47: 4178-4183.

Zoran, D.L. 2002. *The carnivore connection to nutrition in cats.* Journal of American Veterinary Medical Association. Vol. 221: 1559-1567.

APPENDIX A: SUPPLEMENTAL DATA

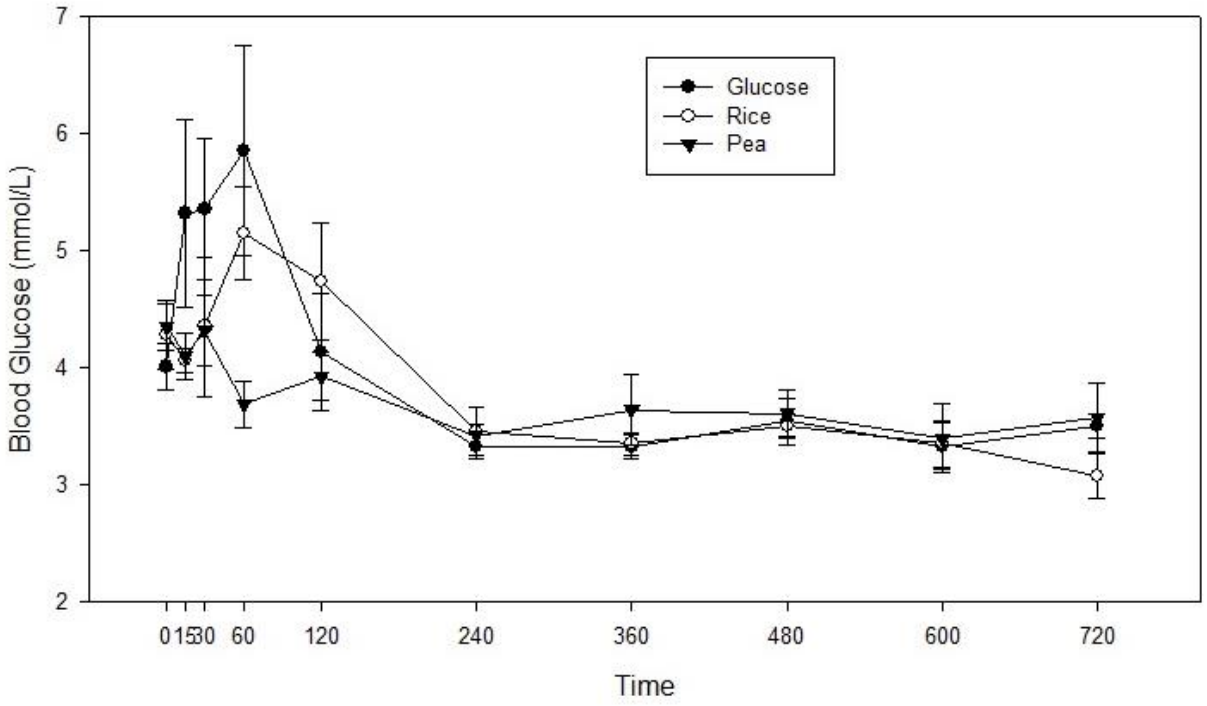


Figure A.1 Cat (n=4) 12-hour time course of postprandial blood glucose levels following single feedings of glucose (15% w/v solution) and two starch sources (rice and pea). Starches were fed in amounts to provide 1 g available carbohydrate per kg bodyweight. Values are shown as mean ± SEM.